Antibacterial activity of a traditional Ayurvedic polyherbal decoction against selected diarrheagenic and non-Diarrheagenic bacterial strains: An *in vitro* study

Munasinghe D.A.L.[1]* and Fernando S.[2]

Abstract

Diarrhea and dysentery remain major public health concerns worldwide, particularly in developing countries. Increasing antimicrobial resistance among enteric pathogens has reduced the effectiveness of conventional antibiotics and necessitated exploration of alternative therapeutic options. Traditional Ayurvedic polyherbal decoctions (*Kashaya*) are widely used in the management of gastrointestinal infections; however, scientific validation of their combined antibacterial efficacy remains limited. The objective of this study was to evaluate the *in vitro* antibacterial activity of a traditional Ayurvedic decoction containing *Plectranthus zeylanicus*, *Zingiber officinale*, *Piper longum*, *Syzygium aromaticum*, and *Trachyspermum ammi* against selected diarrheagenic, dysentery, and non-diarrheagenic bacterial strains. A freshly prepared decoction was evaluated against ten bacterial strains (five diarrheagenic and five non-diarrheagenic) using the agar well diffusion method on Mueller–Hinton agar. Bacterial inocula were standardized to 0.5 McFarland turbidity. Plates were incubated at 37°C for 24 hours, and antibacterial activity was assessed by measuring zones of inhibition. The results revealed that the decoction exhibited antibacterial activity against eight of the ten tested organisms. *Shigella sonnei*, *Shigella flexneri*, *Salmonella typhimurium*, enteropathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae* were sensitive. *Staphylococcus aureus* and enterotoxigenic *Escherichia coli* showed resistant. No zones of inhibition were observed in control plates. The findings provide scientific evidence supporting the traditional use of this Ayurvedic decoction in the management of diarrhea and dysentery. Further quantitative and *in vivo* studies are recommended to determine its clinical applicability.

Keywords: Ayurveda, Polyherbal decoction, Antibacterial activity, Diarrhea, Medicinal plants

Introduction

Diarrheal diseases continue to be a leading cause of morbidity and mortality worldwide, particularly in developing countries. According to the World Health Organization, diarrhoeal disease remains a major contributor to global disease burden, especially among children under five years of age[1].

Bacterial pathogens such as *Escherichia coli*, *Shigella* spp., and *Salmonella* spp. are frequently implicated in diarrheal and dysenteric infections. The rapid emergence of antimicrobial resistance among enteric pathogens has significantly reduced the efficacy of conventional antibiotic therapy[2].

Ayurveda, the traditional system of medicine of India, utilizes polyherbal formulations for the management of gastrointestinal disorders such as *Atisara* (diarrhea) and *Pravahika* (dysentery). Decoctions (*Kashaya*) are aqueous extracts prepared by boiling medicinal plants and are valued for rapid therapeutic action[3]. Several medicinal plants commonly used in such formulations have demonstrated antimicrobial properties individually [4,8].

[1]*Faculty of Indigenous Medicine, University of Colombo, Sri Lanka.*
[2]*Faculty of Meical Sciences, University of Sri Jayewardenepura, Sri Lanka.*

Correspondence: Munasinghe D.A.L., Faculty of Indigenous Medicine, University of Colombo, Sri Lanka. Email: munasinghe@fim.cmb.ac.lk

However, limited scientific evidence exists regarding their combined antibacterial activity in traditional decoction form. Therefore, this study aimed to evaluate the antibacterial efficacy of a selected Ayurvedic polyherbal decoction against diarrheagenic and non-diarrheagenic bacteria under *in vitro* conditions.

Materials and Methods

Plant materials

Leaves of *Plectranthus zeylanicus* L., Rhizomes of *Zingiber officinale* Roscoe, fruit of *Piper longum* L., flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry and seeds of *Trachyspermum ammi* (L.) Sprague were used in the preparation of the decoction. All plant materials were purchased from a local herbal market in Sri Lanka. Botanical authentication was carried out by a qualified taxonomist at the Department of Botany, University of Sri Jayewardenepura. Voucher specimens of each plant were prepared and deposited at the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura for future reference.

Preparation of plant materials

All plant materials were thoroughly washed with distilled water to remove debris and contaminants. The materials were then shade-dried at ambient temperature (approximately 25–30 °C) until a constant weight was achieved.

Preparation of decoction

The decoction was prepared under aseptic conditions at the Nawinna Ayurveda Research Institute by trained technical officers under the supervision of qualified Ayurvedic medical practitioners.

Equal quantities (12g each) of the dried plant materials were weighed, cleaned, and coarsely chopped. The combined plant mixture was placed in an earthenware pot and boiled with 1960mL of distilled water over moderate heat until the volume was reduced to 240mL, following the classical Ayurvedic method[3].

The resulting decoction was filtered through sterile muslin cloth to remove particulate matter. The filtrate was collected as the crude extract and stored in clean amber-colored glass bottles at 4 °C (refrigerator) until further use.

Bacterial strains

Below mentioned diarrheagenic and non-diarrheagenic bacteria were used to *in vitro* study.

Diarrheagenic bacteria

- Enterotoxigenic *Escherichia coli* (ETEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- *Shigella sonnei*
- *Shigella flexneri*
- *Salmonella typhimurium*

Non-diarrheagenic bacteria

- *Pseudomonas aeruginosa*
- *Streptococcus saprophyticus*
- *Enterococcus faecalis*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*

Culture medium

Mueller–Hinton agar was prepared and sterilized at 121°C for 15 minutes.

Preparation of inoculum

Bacterial suspensions were adjusted to 0.5 McFarland standard.

Antibacterial Assay

The agar well diffusion method was performed. Wells (6 mm diameter) were filled with freshly prepared decoction. Ciprofloxacin (10 µg/mL) served as the positive control, while Sterile normal saline served as the negative control. All the test were triplicated. Plates were incubated at 37°C for 24 hours. Zones of inhibition were recorded in millimeters.

Results

The decoction demonstrated antibacterial activity against eight of ten tested organisms (Table 1). The sensitive organisms were: *Shigella sonnei, Shigella flexneri, Salmonella typhimurium, Enteropathogenic E. coli, Pseudomonas aeruginosa, Streptococcus saprophyticus, Enterococcus faecalis* and Streptococcus *pneumoniae*.

Table 1: Antibacterial activity of plant decoction against selected bacterial strains (agar well diffusion assay)

Organism	Decoction (mm)	Positive control (mm)	Negative control (mm)	Interpretation
Shigella sonnei	14.0 ± 0.6	26.2 ± 0.9	0	Sensitive
Shigella flexneri	15.1 ± 0.6	27.3 ± 1.6	0	Sensitive
Salmonella typhimurium	14.1± 0.5	25.0± 1.1	0	Sensitive
Enteropathogenic *E. coli*	13.2 ± 0.7	24.4± 1.2	0	Sensitive
Pseudomonas aeruginosa	12.3± 0.6	22.0± 1.5	0	Sensitive
Streptococcus saprophyticus	15.4± 0.8	24.2± 1.4	0	Sensitive
Enterococcus faecalis	14.4± 0.6	20.3± 0.9	0	Sensitive
Streptococcus pneumoniae	15.1± 0.6	23.5± 1.4	0	Sensitive
Staphylococcus aureus	0	21.4± 1.8	0	Resistant
Enterotoxigenic *E. coli*	0	24.2± 1.9	0	Resistant

No inhibition was observed in negative control plates. All tested bacterial strains demonstrated susceptibility to ciprofloxacin (10 µg/mL), as evidenced by clear zones of inhibition.

Discussion

The present findings demonstrate broad-spectrum antibacterial activity of the tested polyherbal decoction. Sensitivity of *Shigella* spp., *Salmonella typhimurium*, and EPEC supports its traditional use in diarrhea and dysentery management.

The antimicrobial activity may be attributed to synergistic phytoconstituents. *Zingiber officinale* contains gingerols and shogaols[4]. *Piper longum* contains piperine, known for antibacterial and bioavailability-enhancing effects[5,6]. *Syzygium aromaticum* is rich in eugenol[7], and *Trachyspermum ammi* contains thymol[8].

Resistance observed in *Staphylococcus aureus* and ETEC suggests possible intrinsic resistance mechanisms and highlights the need for minimum inhibitory concentration (MIC) studies and phytochemical analysis.

Conclusion

This study provides preliminary scientific validation for a traditional Ayurvedic decoction used in the management of diarrhea and dysentery. The formulation exhibited antibacterial activity against a majority of tested pathogens. Further quantitative, phytochemical, and in vivo studies are recommended to establish clinical efficacy.

References

1. World Health Organization. (2017). Diarrhoeal disease. Geneva: World Health Organization.
2. Ventola CL. (2015). The antibiotic resistance crisis. *Pharmacy and Therapeutics*. 40:277–283.
3. Sharma PV. (2013). *Dravyaguna Vijnana*. Varanasi: Chaukhambha Bharati Academy.
4. Ali BH, Blunden G, Tanira MO, Nemmar A. (2008). Some phytochemical and pharmacological properties of ginger. *Food and Chemical Toxicology*. 46:409–420.
5. Srinivasan K. (2007). Black pepper and piperine: A review. *Critical Reviews in Food Science and Nutrition*. 47:735–748.
6. Meghwal M, Goswami TK. (2013). Piperine: A review. *Comprehensive Reviews in Food Science and Food Safety*. 12:443–459.
7. Chaieb K, Hajlaoui H, Zmantar T, et al. (2007). Chemical composition and biological activity of clove essential oil. *Phytotherapy Research*. 21:501–506.
8. Bairwa R, Sodha RS, Rajawat BS. (2012). *Trachyspermum ammi*: A review. *Pharmacognosy Reviews*. 6:56–60.