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Research Article

IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *DENIMBA DEBATU KASHAYA*, A TRADITIONAL SRI LANKAN POLYHERBAL DECOCTION

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ABSTRACT

Denimba debatu kashaya is a renowned polyherbal formulation from the traditional Sri Lankan text *Vatikaprakaranaya*, where it is indicated as an *Anupana kashaya* (adjuvant decoction). In contemporary practice, it is widely used for managing infectious diseases. This study was designed to scientifically evaluate the *in vitro* antimicrobial potential of *Denimba debatu kashaya*, both with and without its traditional additives (*Prativapa dravya*), against common pathogens. The decoction was prepared according to classical guidelines and tested against *Staphylococcus aureus* (ATCC™ 25923), *Escherichia coli* (ATCC™ 25922), and a clinical isolate of *Candida albicans* using the agar well diffusion method. Augmentin (100µg/mL) and Itraconazole (100µg/mL) were used as positive controls. The results demonstrated that the *Kashaya* samples did not produce any measurable zone of inhibition (ZOI) against the tested microbes. However, a slight reduction in bacterial growth was observed near the sample wells. This suggests that the standard preparation may possess an intermediate or weak effect, or its therapeutic efficacy in traditional settings may be attributed to mechanisms other than direct antimicrobial action, such as immunomodulation. Further studies using higher concentrations are recommended to fully explore its antimicrobial properties.

INTRODUCTION

The Siddha and Ayurveda systems of medicine, deeply rooted in South Asian culture, emphasize holistic healing through formulations derived from natural sources[1]. In Sri Lanka, this traditional knowledge is preserved in classical texts like the *Vatikaprakaranaya*, which provides detailed instructions for preparing medicines like pills (*Guli*) and pastes (*Kalka*)[2]. These are often administered with an *Anupana* (adjuvant) to enhance therapeutic outcomes[3].

One of the most prominent formulations mentioned is *Sitarama vaṭi*, prescribed for ailments like *Jwara* (fever) and *Atisara* (diarrhoea)[4].

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It is often administered with "*Denimba debatu anupana kashaya*," a decoction prepared from eight potent herbs.

Today, traditional practitioners frequently use this *kashaya* as a standalone remedy for various infections based on empirical evidence and knowledge of the pharmacodynamics of its individual ingredients [5]. Despite its widespread use, there is a significant lack of scientific validation for the antimicrobial claims of the complete formulation. This study aims to scientifically evaluate the *in vitro* antibacterial and antifungal activity of the traditional Sri Lankan polyherbal formulation, *Denimba Debatu Kashaya*, thereby addressing the existing gap in scientific evidence supporting its traditional use. The objectives of this research are to conduct a systematic *in vitro* assessment of the antibacterial and anti-fungal activities of *Denimba Debatu Kashaya* against selected pathogenic bacterial and fungal strains, and to compare the results with standard reference drugs in order to validate its traditional therapeutic claims.

MATERIALS AND METHODS

Source and Authentication of Raw Drugs

The eight plant ingredients (Table 1 and Fig. 1) and the *Prativapa dravya* (*Hingu* and *Saindhava lavana*) were procured from a licensed herbal drug store. All raw materials were validated by an expert at the Department of Dravyaguna Vignana, Faculty of Indigenous Medicine, Gampaha Wickramarachchi University of Indigenous Medicine, Yakkala, prior to the commencement of the research.

Table 1: Composition of *Denimba debatu kashaya*

S.No.	Botanical Name	Family	Part Used
1	*Azadirachta indica* A. Juss.	Meliaceae	Bark
2	*Munronia pinnata* (Wall.) W. Theob.	Meliaceae	Whole plant
3	*Solanum melongena* L.	Solanaceae	Root
4	*Solanum virginianum* L.	Solanaceae	Whole plant
5	*Oroxylum indicum* (L.) Kurz	Bignoniaceae	Bark
6	*Cissampelos pareira* L.	Menispermaceae	Whole plant
7	*Crateva nurvala* Buch-Ham.	Capparaceae	Bark
8	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome

Fig. 1: Purchased materials *(a) Azadirachta indica (b) Munronia pinnata (c) Solanum melongena (d) Solanum virginianum (e) Oroxylum indicum (f) Cissampelos pareira (g) Crateva nurvala (h) Zingiber officinale (i) Saindhava lavana (j) Hingu*

Method of Preparation

The decoction was prepared according to the standard *Kwatha paribhasha* (decoction guidelines) of Ayurveda [8]. Two samples were prepared: one without additives and one with the addition of *Hingu* (2g) and *Saindhava lavana* (2g) [9]. The prepared decoctions (Fig. 2) were filtered, stored in sterile containers at 4°C, and used within two weeks [6].

Fig. 2: The prepared decoctions

Antimicrobial Assay

The *in vitro* antimicrobial activity was assessed using the agar well diffusion method[6,12]. The test organisms were *Staphylococcus aureus* (ATCC™ 25923), *Escherichia coli* (ATCC™ 25922), and a clinical isolate of *Candida albicans*. Standardized microbial inocula (0.5 McFarland) were spread on Nutrient Agar (for bacteria) and Potato Dextrose Agar (for fungi) plates. Wells of 6mm diameter were created, into which 50µL of the test samples were loaded. Augmentin (100µg/mL) and Itraconazole (100µg/mL) were used as positive controls[10,11], and sterile distilled water was used as a negative control. The plates were incubated for 24 hours, after which the zone of inhibition (ZOI) was measured.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean±SD. The data were analysed using one-way ANOVA with Tukey's

post-hoc test (IBM SPSS Statistics 22), with $P<0.05$ considered statistically significant.

RESULTS AND DISCUSSION

The antimicrobial screening revealed that neither the *Denimba debatu kashaya* sample without additives nor the sample with *Prativapa dravya* produced any measurable zone of inhibition against *S. aureus*, *E. coli*, or *C. albicans*. The positive controls, however, showed significant activity, with mean ZOI values of 27.33±0.24 mm (*S. aureus*), 10.33±0.24 mm (*E. coli*), and 15.33±0.24 mm (*C. albicans*). Although no clear zone of inhibition was present, careful observation of the antibacterial plates showed that the bacterial growth immediately surrounding the wells with the *Kashaya* samples was slightly less dense than in other areas of the plate (Fig. 3).

Table 2: Zone of inhibition (mm) related to the tested microbial strains

Microorganism	Positive Control (Mean ± SD)	Negative Control	*Kashaya* (without *Prativapa*)	*Kashaya* (with *Prativapa*)
Staphylococcus aureus	27.33 ±0.24	ND	ND	ND
Escherichia coli	10.33 ±0.24	ND	ND	ND
Candida albicans	15.33 ±0.24	ND	ND	ND

ND = Not Detected

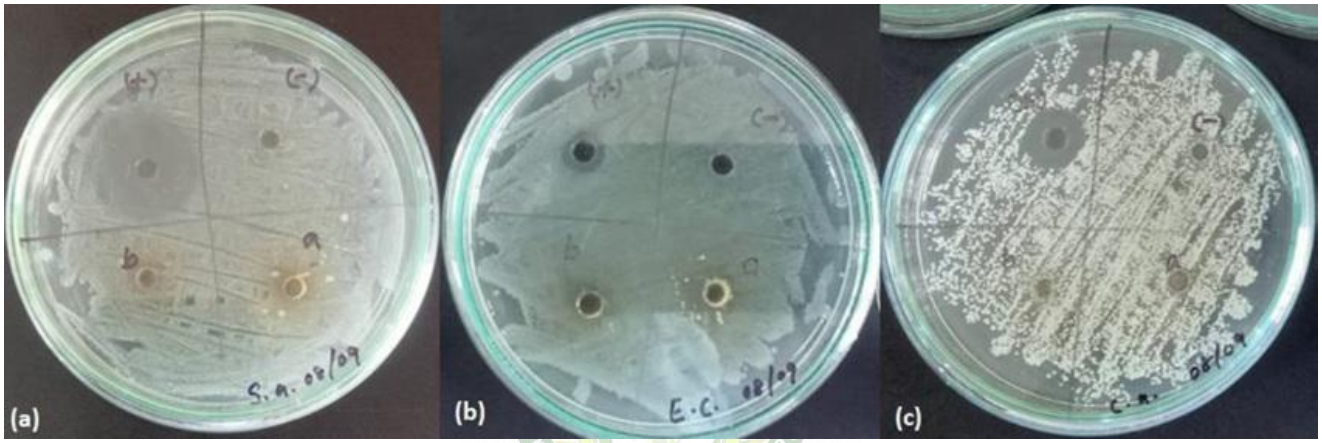

Fig. 3: Antimicrobial assay plates for (a) *S. aureus*, (b) *E. coli*, and (c) *C. albicans*

The physicochemical parameters of this study demonstrate that the standard preparation of *Denimba debatu kashaya* does not possess significant direct antimicrobial properties *in vitro*. The lack of a clear zone of inhibition against both Gram-positive and Gram-negative bacteria, as well as the tested fungus, indicates that the concentration of active antimicrobial compounds in the aqueous extract is likely below the minimum inhibitory concentration required to halt microbial growth.

However, the traditional and continued use of this decoction for treating infections cannot be disregarded. The subtle reduction in bacterial growth observed near the sample wells may suggest a very mild bacteriostatic effect. More importantly, the therapeutic efficacy of many herbal formulations is not solely dependent on direct antimicrobial action. The clinical benefits could be due to other pharmacological properties such as immunomodulatory, anti-inflammatory, or antipyretic effects, which help the body's own defence mechanisms to fight off infection. The combination of phytoconstituents from eight different herbs could work synergistically to modulate the host response rather than directly killing the pathogens. This study highlights the need for a broader approach to validating traditional medicines, one that goes beyond simple antimicrobial screening.

CONCLUSION

The standard aqueous preparation of *Denimba debatu kashaya* demonstrated no significant *in vitro* antimicrobial activity against the selected pathogens. These findings support the need for further pharmacological and clinical studies to establish its therapeutic efficacy and mechanism of action, which may involve immunomodulation or other host-centred effects. Future research should also explore different extraction methods and higher concentrations to fully assess its potential.

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