

Antimicrobial activity of sugar-based semisolid polyherbal ayurvedic formulation: Chyawanprash

Abstract

Objective: This study evaluated the antimicrobial activity of chloroform (CHCl₃) extracts of chyawanprash (Dabur India Ltd., Uttar Pradesh, India) and hydrolyzed chyawanprash against *Escherichia coli* and *Staphylococcus aureus* on nutrient agar media using cup plate method. **Materials and Methods:** CHCl₃ extracts of chyawanprash were prepared directly as well as by previously hydrolyzing with dilute HCl. Three test groups of 10, 20, and 50 mg/ml were prepared for each CHCl₃ extract, whereas ampicillin 20 mg/ml was used as standard group. Further, six agar plates were prepared for each group, i.e., three for *E. coli* and three for *S. aureus*. Cups were bored on each plate with the help of steel cup borer and cups on each plate were marked for identification. Extracts of different concentrations (i.e., 10, 20, and 50 mg/ml) were poured into cups and all plates were then incubated at 37°C for 48 h. After incubation, plates were observed for bacterial growth and zone of inhibition were measured. **Results:** Results showed that both CHCl₃ as well as hydrolyzed CHCl₃ extract of chyawanprash showed concentration-dependent antimicrobial activity. The largest zone of inhibition was observed at the concentration of 50 mg/ml of both CHCl₃ extracts of chyawanprash (12.7 ± 1.5 mm for *E. coli* and 15.0 ± 1.0 mm for *S. aureus*) and hydrolyzed chyawanprash (14.3 ± 0.6 mm for *E. coli* and 16.3 ± 0.6 mm for *S. aureus*). **Conclusion:** Chyawanprash possesses promising potential for use as an antimicrobial agent.

Key words:

Antimicrobial activity, chyawanprash, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Chyawanprash is an ayurvedic formulation frequently consumed in India. Chyawanprash is a comprehensive herbal tonic, prepared from around 50 herbs. *Emblica officinalis* is the basic ingredient of chyawanprash. It has shown to possess good anti-amnesic and cognition enhancing properties.^[1,2] Chyawanprash is also reported to reduce postprandial glycemia in the oral glucose tolerance test and blood cholesterol level.^[3] Administration of chyawanprash has been shown to prevent cisplatin-induced acute renal toxicity.^[4] This study was undertaken to explore the antimicrobial effects of chyawanprash of Dabur India Ltd., which is being marketed in India.

Mohammad Ahmed Khan, Satyajyoti Kanjilal¹,

Arun Gupta¹, Sayeed Ahmad

Bioactive Natural Product Laboratory,

Department of Pharmacognosy and Phytochemistry,

Faculty of Pharmacy, Hamdard University, New Delhi,

¹Dabur Research and Development Center, Sahibabad,

Ghaziabad, Uttar Pradesh, India

Address for correspondence:

Dr. Sayeed Ahmad,

Bioactive Natural Product Laboratory, Faculty of Pharmacy,

Hamdard University,

New Delhi - 110 062, India.

E-mail: sahmada_jh@jamiahamdard.ac.in

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Materials and Methods

Preparation of extracts

Preparation of chloroform extract of chyawanprash

Twenty gram of formulation was extracted by suspending in 50 ml of water. Further, the aqueous extract was fractionated with 100 ml of chloroform (CHCl₃) by putting the same in separating funnel for 30 min with occasional shaking and repeating the process thrice to ensure complete extraction. All the three extracts were pooled, evaporated to dryness on water bath. This residue obtained was dissolved in dimethyl sulfoxide (DMSO) to get different concentrations, which was used (0.1 ml each) for antimicrobial activity against *Escherichia coli*/*Staphylococcus aureus* on nutrient agar media by cup plate method.

Preparation of chloroform extract (previously hydrolyzed with dilute HCl)

Twenty gram of chyawanprash was suspended in 50 ml dilute HCL followed by heating it on reflux condenser for 20 min. This was then extracted with CHCl₃ using separating funnel after cooling as mentioned above. Pooled extracts were evaporated to dryness and residue obtained was dissolved in DMSO to get different concentrations which were used (0.1 ml each) for antimicrobial activity against *E. coli*/*S. aureus* on nutrient agar media by cup plate method.

Preparation of seeded agar plates

Preparation of nutrient agar medium

Twenty gram of nutrient agar medium was dissolved in 1 L of double distilled water and heated for 20 min. After heating, it was poured into five conical flasks (250 ml), flasks were cotton plugged, and plug was covered with aluminum foil and sterilized by autoclaving.

Preparation of base layer

Sterilized nutrient agar medium was poured into sterile petri plates under aseptic condition (laminar flow) and allowed to cool for preparation of base layer.

Inoculation of plates

One ml of bacterial culture (*E. coli* or *S. aureus*) was mixed with 3 ml of sterile agar medium and thoroughly mixed. This liquid was then aseptically poured over base layer and allowed to cool to form seed layer. After preparation, agar plates were marked from the bottom side for identification of different cups and plates as well as bacteria.

Total six plates were prepared for each group and divided as three for *E. coli* and three for *S. aureus*. Cups were bored on each plate with the help of steel cup borer and cups on each plate were marked for identification. Extracts of different concentrations were poured into cups of both the bacteria.

All plates will then be incubated at 37°C for 48 h. After incubation, plates were observed for bacterial growth and zone of inhibition was measured.

Results and Discussion

Plates were prepared, inoculated, incubated, and successfully observed. Extracts of chyawanprash showed antimicrobial activity as evident from the observation of zone of inhibition. Results showed that both CHCl₃ as well as hydrolyzed CHCl₃ extracts of chyawanprash showed concentration-dependent antimicrobial activity [Table 1]. The largest zone of inhibition was observed at the concentration of 50 mg/ml of both CHCl₃ extracts of chyawanprash (12.7 ± 1.5 mm for *E. coli* and 15.0 ± 1.0 mm for *S. aureus*) and hydrolyzed chyawanprash (14.3 ± 0.6 mm for *E. coli* and 16.3 ± 0.6mm for *S. aureus*). Hydrolyzed extract showed highest activity at 50 mg/ml concentration viz., 84% of standard [Table 2]. It may be postulated that the antimicrobial activity of CHCl₃ extract of chyawanprash may be attributed to the presence of medium polar and some nonpolar constituents of formulation. Whereas higher activity of hydrolyzed

Table 1: Zone of inhibition (Z) of different extracts of Chyawanprash (n=3)

Sample	Concentration (mg/ml)	Bacteria	Zone of inhibition (mm)			
			Plate 1	Plate 2	Plate 3	Average
Chloroform extract	10	<i>E. coli</i>	9	10	8	9
		<i>S. aureus</i>	12	12	12	12
	20	<i>E. coli</i>	12	11	12	11.7
		<i>S. aureus</i>	12	13	11	12
	50	<i>E. coli</i>	13	11	14	12.7
		<i>S. aureus</i>	16	15	14	15
Hydrolyzed chloroform extract	10	<i>E. coli</i>	10	10	10	10
		<i>S. aureus</i>	12	11	13	12
	20	<i>E. coli</i>	12	12	14	12.7
		<i>S. aureus</i>	13	13	13	13
	50	<i>E. coli</i>	14	15	14	14.3
		<i>S. aureus</i>	16	17	16	16.3
Standard (ampicillin)	20	<i>E. coli</i>	17	19	15	17
		<i>S. aureus</i>	23	29	26	26
Blank		No activity was observed with any bacteria				

E. coli – *Escherichia coli*; *S. aureus* – *Staphylococcus aureus*

Table 2: Percentage activity of different extracts compared to standard

Bacterias	Percentage activity of chloroform extract (mg/ml)			Percentage activity of hydrolyzed chloroform extract (mg/ml)		
	10	20	50	10	20	50
<i>E. coli</i>	52.94	68.8	74.7	58.8	74.7	84.11
<i>S. aureus</i>	46.15	46.15	57.7	46.15	50	62.7

E. coli – *Escherichia coli*; *S. aureus* – *Staphylococcus aureus*

CHCl₃ extract may be because of the presence of some aglycones of glycosides present in different constituents of plant formulation.

Conclusion

Chyawanprash has a good antimicrobial activity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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