Research Article

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ANTIMICROBIAL ANALYSIS OF NALPAMARADI TAILA BATH SOAP: *IN VITRO* STUDY

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ABSTRACT

The procedures mentioned for skin care, as part of daily routine, are udvartana (powder massage), abhyanga (oil massage), and snana (bathing). Udvartana promotes skin health by lowering Kapha and liquefying fat. According to Bhavaprakasha, Snana removes mala and sweda and can be performed with amlaka water, which helps to reduce wrinkles and grey hairs. However, due to a hectic lifestyle and a lack of time, snana is performed with hot water, gram flour, turmeric, or, more commonly, soaps and moisturisers after bathing. In Sahasrayogatailaprakaran, Nalpamaradi taila treats itching, scabies, Visarpa (erysipelas), and Kushta (skin disease/leprosy). Nalpamaradi taila is used in this study to make soap, where studies were carried out to check its antimicrobial activity. The study was carried out in two phases. In the first phase, samples were taken from the volunteers from contaminated hands after washing with Nalpamaradi soap immediately and at half-hour intervals. The sample was cultured and observed, which showed a reduction in microbial growth bacteria and fungus. In phase two, Nalpamaradi taila soap was tested for its effect on *C.albicans, E.coli, P.aeruginosa* and *S.aureus*. No growth of the above microorganisms was observed after treatment with Nalpamaradi soap. The sample was cultured and observed, revealing a decrease in microbial growth bacteria and fungus. Nalpamaradi soap was tested in phase 2 for its effect on *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Following treatment with Nalpamaradi taila soap, no growth of the microorganisms above was observed. This proves the antimicrobial properties of Nalpalmaradi soap.

Keywords: Nalpamaradi taila, Soap, Skin, Antimicrobial, Krimighna

INTRODUCTION

Udvartana (powder massage), abhyanga (oil massage), and snana (bathing) are mentioned with various benefits in Ayurveda as part of daily routine. Udvartana helps to enhance and maintain skin health. It also reduces Vata and Kapha aggravation, liquefies fat, and stabilises the body. It also increases circulation, promotes metabolic activity, and improves skin complexion. Udvartana is used to treat various diseases based on the prakriti of the patient. Several studies have shown that udvartana is effective in a variety of diseases. Triphala, Kola, Kulatha, and other drugs are very commonly used to treat udvartana. Daily practice of abhyanga improves skin appearance, alleviates Vata disorders, and increases tolerance to adversity and physical strain. Tila taila is a popular oil for abhyanga. Snana is one of the daily regimens that removes mala and sweda and assists individuals in achieving health and longevity. According to Bhavprakasha, even rubbing the entire body with a cloth immediately after bathing improves skin colour and complexion, removes itching, and cures various skin illnesses. Bathing in Amlaka water, according to Bhavaprakasha, eliminates wrinkles and grey hairs and extends one's life by hundreds of years. However, due to a busy lifestyle and a lack of time, most people cannot perform all Ayurvedic procedures. As a result, snana is performed with hot water, gram flour, turmeric, or, more commonly, soaps. Creams and moisturisers are applied after bathing. According to Indian soap-making standards, the mass loss due to mushing should be 15 g/50 cm^2, free caustic alkali 0.05%, free carbonated alkali 1%, TFM min 40%, and it should pass the tests for grittiness, cracking, and cleaning efficacy. Soaps with varna properties are useful for skin care and can be made by adding varnya medications[1].

This study uses Nalpamaradi taila to make soap using Nalpamaradi taila, which provides all of the benefits listed above. Nalpamaradi taila, mentioned in Sahasrayogatailaprakaran, is used in itching, scabies, Visarpa (erysipelas), and Kushta (skin disease)[2].

The ingredients of Nalpamaradi taila are as follows. (Table 1).

Table 1: Ingredients of Nalpamaradi taila

Sl	Name of drugs	Quantity
1	Nyagrodha *Ficus benghalensis* L.	
2	Udumbhara *Ficus racemosa* Linn.	
3	Ashwattha *Ficus religiosa* Linn.	
4	Flaksha *Ficus Lacor*	
5	Haritaki *Terminalia chebula* Retz	
6	Vibhitaki *Terminalia Bellirica*	
7	Amalaki *Phyllanthus emblica* L	
8	Chandana *Pterocarpus santalinus* Linn.	
9	Ushira *Vetiveria zizanioides* Linn	
10	Kushtha *Saussurea lappa*	
11	Manjistha *Rubia cordifolia* L	
12	Coram *Kaempferia galanga* L	
13	Agaru *Aquilaria agallocha* Roxb.	
14	Haridra Juice *Curcuma longa* L	
15	Parpata Juice *Fumaria parviflora* Lam	
16	Tilataila *Sesamum indicum* L.	4 parts

MATERIALS AND METHOD

Nalpamaradi taila is prepared with the above ingredients at the teaching pharmacy of the Institution Sri Kalabyraveshwara Swamy Ayurvedic Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India.

Soap was prepared using Nalpamaradi taila, coconut oil, olive oil, distilled water, 100 percent pure lye (Sodium hydroxide) and drops of essential oils, i.e., combined Bergamot and cedarwood, for aroma. Nalpamaradi taila taken in a cooker. Once it started melting, the lye was slowly added to the lye to the water using all the necessary safety gear. Using a spatula, the solution was stirred. The lye was kept aside for cooling for 15 to 20 minutes. Melted coconut oil, olive oil and Nalpamaradi taila were mixed and stirred well. During preparation, the temperature of the oils was checked using the candy thermometer. Once the oils reached 120 to 130 °F (49 to 54 °C), the immersion blender was placed on the side of the heating vessel. Lye was poured gently to avoid splashing and stirred slowly, moving in circles by keeping the blender immersed to prevent air bubbles. Continued blending and stirring for 10 to 15 minutes until the soap reached trace and the solution thickened and resembled pudding. The heating vessel was covered and cooked on low flame for 50 minutes. Turned off the heat, and the vessel was allowed to cool until the mixture dropped below 180 °F (82 °C). The essential oils were added and mixed well.

The mixture was poured into the soap mould, and a spatula smoothened the top. The surface was tapped to eliminate the air bubbles. The prepared soap was packed and labelled. Following the preparation of the soap, it was sent for antimicrobial study. The drugs in the Nalpamaradi taila possess antimicrobial properties, considering this Nalpamaradi taila was used as a base for preparing soap.

After receiving approval from the Institutional Ethics Committee (SKAMCH & RC/ IEC/003/2020), healthy volunteer Group D workers of the Institution with no skin abrasions, wounds, or infections on the palms and hands and aged 18-60 years were included in the study. Volunteers who refused to participate in the study or had previously experienced sensitive reactions when certain chemicals were applied to their body surface were excluded from the trial. The volunteers with the most contaminated hands were asked to wash their hands with soap, and swab samples were taken immediately and at half-hour intervals from the healthy volunteers and, after that, were inoculated and cultured.

Total Count Analysis

Luria Bertani (LB) agar media (Tryptone 10 g, sodium chloride 10 g, yeast extract 6 g, agar 15 g, distil water 1000 mL) 150 mL was prepared and autoclaved at 121 °C for 15 minutes. 100 µL of the sample was poured into the sterilised Petri plate and spread thoroughly; approximately 20 mL of the LB agar was poured into the plate, allowed for solidification (pour plate method) and incubated at 37 °C for 24 hours. After 24 hours, the plates were observed and tabulated as colony-forming units.

Table 2: Colony forming Units/mL

Sample	Colony forming units /mL
Before	1.84×10^3
Immediate	1.04×10^3
5 minutes	1.06×10^3
15 minutes	1.16×10^3
30 minutes	1.18×10^3

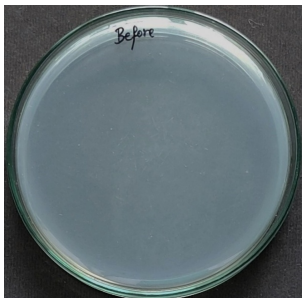


Figure 1

Figure 2

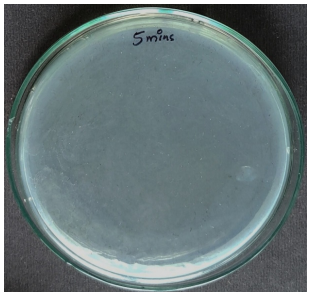


Figure 3

Figure 4

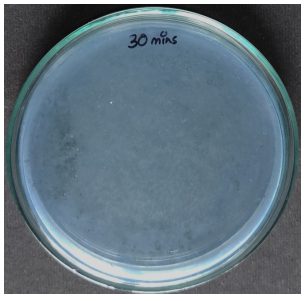


Figure 5

In vitro, soap showed an insignificant reduction in CFU immediately, after 5 minutes, after 15 minutes, and after 30 minutes. (Table 2, Figure 1 to 5)

MICROBIAL ANALYSIS

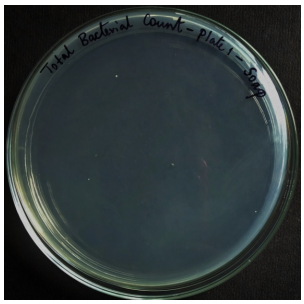
The soap sample was dissolved in 1 mL of distilled water and tested for the microbial limit test.

Total Count Analysis

Luria Bertani (LB) agar media (Tryptone 10 g, sodium chloride 10 g, yeast extract 6 g, agar 15 g, distil water 1000 mL) 50 mL was prepared and autoclaved at 121 °C for 15 minutes. 100 µL of the sample was poured into the sterilised Petri plate and spread thoroughly; approximately 25 mL of the LB agar was poured into the plate, allowed for solidification (pour plate method), and incubated in an incubator at 37 °C for 24 hours. After 24 hours, the plates were observed, and colony-forming units were tabulated.

Table 3: Colony forming Units/mL

Sample	Colony forming units /mL
Plate 1	0.49×10^3
Plate 2	0.38×10^3



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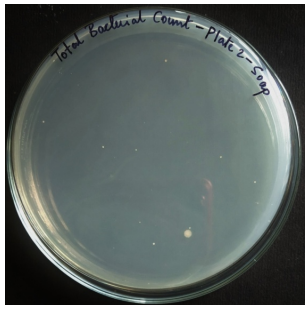


Figure 7

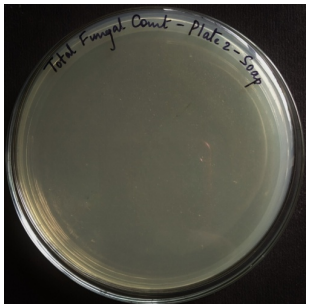
The bacterial colony reduced more quickly (Plate 1) than after 30 minutes (Plate 2) of washing, which was reduced significantly ((Table 3, Figure 6, 7)).

Total Fungal Count Analysis

Potato Dextrose Agar (PDA) media (potato 200 g, dextrose 20 g, agar 20 g, distilled water 1000 ml) 50 ml was prepared by boiling 10 g of potato in 30 ml distilled water and filtered, 1 g dextrose, 1 g agar was added into the filtrate and the volume was made upto 100 ml with distilled water. Autoclaved at 121 °C for 15 minutes. The soap sample 100 µL was poured into the sterilised Petri plates respectively, and approximately 25 mL of the PDA media was poured into the respective plates, allowed for solidification (pour plate method) and incubated for 72 hours at 25 °C. After 72 hours, the plates were observed, and CFU was tabulated.

Table 4: Colony forming Units/mL

Sample	Colony forming units /mL
Plate 1	-
Plate 2	-



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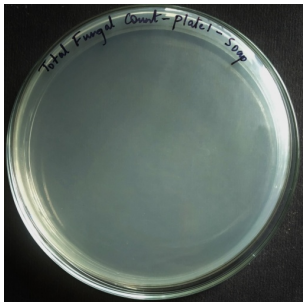


Figure 9

The fungal growth was not observed in the sample collected immediately (Plate 1) and in the sample after 30 minutes (Plate 2)of washing(Table 4, Figure 8, 9).

Antimicrobial Activity

Well diffusion method to check the Minimum Inhibition Concentration (MIC)

The soap sample was tested in duplicates for their MIC property against organisms (*Candida albicans, E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*).

Culture Media Preparation for Fungus

Potato Dextrose Broth (PDB: potato 200 g, dextrose 20 g, distilled water 1000 mL) 30 mL was prepared in Erlenmeyer flask by boiling 6 g of potato in 30 mL distilled water and filtered. 0.6 g of dextrose was added into the filtrate, and the final volume was made up to 30 ml with distilled water respectively and autoclaved at 121°C for 15 minutes.

Candida albicans (MTCC 3958) was inoculated and incubated at 25°C for 72 hours.

Culture Media Preparation for Bacteria

Luria Bertani (LB) broth (tryptone 10 g, sodium chloride 10 g, yeast extract 6 g, distilled water 1000 mL) 30 mL was prepared in 3 respective Erlenmeyer flasks by adding tryptone 0.3 g,

sodium chloride 0.3 g, yeast extract 0.18 g, distilled water 30 mL and autoclaved at 121°C for 15 minutes.

E.colistrain (MTCC 433), *Pseudomonas aeruginosa strain* (MTCC 2453) and *Staphylococcus aureus strain* (MTCC 96) were inoculated respectively in 30 mL of sterilised sterilised LB broth flasks and incubated at 37 ^{0}C for 24 hours. Later, 5 mL of broth cultures were centrifuged at 6000 rpm for 10 minutes, the supernatant was discarded, and the pellets were dissolved in 1% ($^w/_v$) saline respectively and adjusted to absorbance 1.000 at 600 nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

Sample Preparation

100 mg of soap sample was dissolved in 1 mL of distilled water. Different aliquots of the sample were prepared by pipetting 10 µL (1 mg), 20 µL (2 mg), 30 µL (3 mg), and 40 µL (4 mg) and the final volume was made up to 50 µL by adding distilled water.

Media preparation for MIC

For the fungal plate: Potato Dextrose Agar media 150 mL was prepared (PDA: potato 200 g, dextrose 20 g, agar 20 g, distilled water 1000 mL). Potato 10 g was boiled in 30 mL of distilled water and filtered; the final volume was 50 mL with distilled water. 1 g dextrose 1 g agar was added and autoclaved at 121°C for 15 minutes.

For bacterial plate: Luria Bertani (LB) agar media (tryptone 10 g, sodium chloride 10 g, yeast extract 6 g, agar 20 g, distilled water 1000 mL). 150 mL was prepared in Erlenmeyer flasks by adding tryptone 1.5 g, sodium chloride 1.5 g, yeast extract 0.9 g, agar 3 g, and distilled water 150 ml respectively and autoclaved at 121°C for 15 minutes.

Platting for MIC against organisms

Approximately 25 mL of the media (PDA and LB agar) was poured into the sterilised Petri plates and allowed to solidify. 200 µL *Candida albicans* and prepared inoculum *(E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus)* were poured respectively on an agar plate and spread thoroughly using a plate spreader. Five wells measuring 0.6 cm were made in each plate using the borer, and 50 µL of a prepared sample containing 1 mg, 2 mg, 3 mg and 4 mg were loaded into the respective wells, and 50 µL of DMSO was loaded in the middle well as control blank.

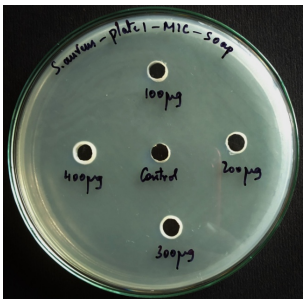
The bacterial plates were incubated at 37 °C for 24 hours, and the fungal plates were incubated at 25 C for 72 hours. Later, the zone of inhibition was recorded in mm (millimetre).[3,4]

RESULTS

Minimum Inhibition of Concentration of Sample

Table 5: MIC of soap sample against Pathogens

Organism	Zone of inhibition of soap sample against *Pathogens* in mm							
	Concentration in µg							
	1 mg		2 mg		3 mg		4 mg	
Plate	1	2	1	2	1	2	1	2
C.albicans	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-
P.aeruginosa	-	-	-	-	-	-	-	-
S.aureus	-	-	-	-	-	-	-	-



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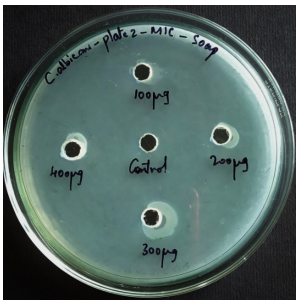

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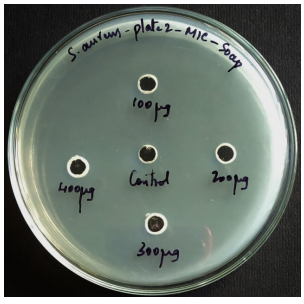

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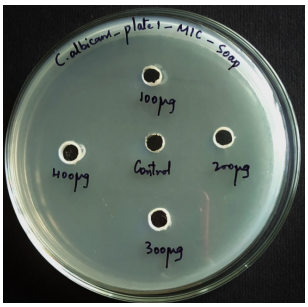

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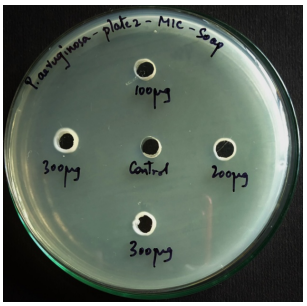

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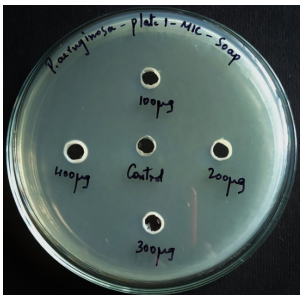

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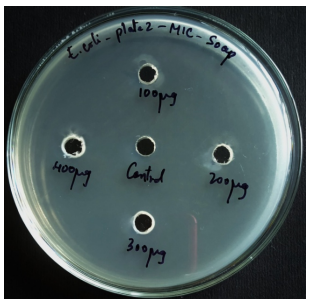

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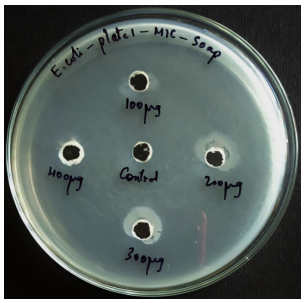

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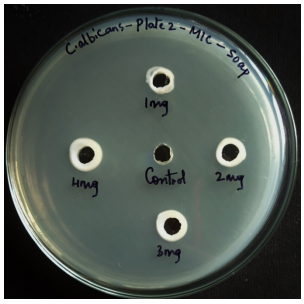

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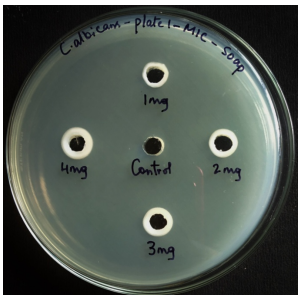

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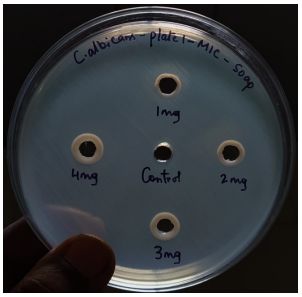

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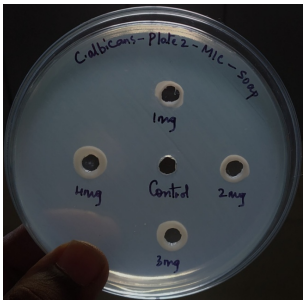

Figure 21

Pathogens *C. albicans, E. coli, P. aeruginosa,* and *S. aureus* were reduced entirely after treatment with the prepared soap sample (Table 5, Figures 10 to 21).

DISCUSSION

As mentioned above, Nalpamaradi taila is useful in itching, scabies, Visarpa (erysipelas), and Kustha (skin disease). Most drugs in the Nalpamaradi taila contain tridoshashamaka quality, which is beneficial in maintaining skin health. Most drugs have actions such as Kushtahara, Twakrogahara (useful in skin diseases), varna vikara (pigmentation disorders), Kandu (itching), and so on[5].

Nyagrodha, Udumbara, Ashwattha and Plaksha are four drugs among the panchavalkala: these process kashaya rasa and Kapha Pittahara actions. The aqueous and alcoholic extracts contain phytochemical constituents of anti-inflammatory, antibacterial, anti-parasitic, antifungal and wound healing properties.

Haritaki, Vibhitaki and Amalaki constitute the Triphala, which is beneficial for skin conditions because of its Kapha and Pitta-reducing action. Triphala contains the Krimighna property. Tikta, madhura rasa, and sheeta veerya are found in Chandana and Ushira; hence, it is beneficial in Pitta and Kapha vikara. Chandana additionally shows krimihara action.

Kushtha contains kashaya, madhura rasa, and ushna veerya, which can be used to treat skin diseases. Manjista possesses kashaya, tikta, and madhura rasa, undergoes madhura vipaka, has raktashodhaka action, and is indicated in most skin diseases. Methanol extract of Manjista demonstrated antibacterial activity against all three gram-positive bacteria tested (*B. subtilis, E. faecalis,* and *S. aureus*) as well as four gram-negative bacteria (*A. baumannii, E. aerogenes, P. mirabilis,* and *P. aeruginosa*) and antifungal activity against Candida albicans, While the methanol extract of *R. cordifolia* root did not show antibacterial activity against three gram-negative bacteria (E. *coli, K. pneumonia,* and *S. enteritidis*).[6]

Coram and Agaru also possess Krimihara action due to ushna veerya and katu-tikta rasa. The formation of an inhibition zone indicated that Coram could inhibit the growth of pathogenic fungi and bacteria. According to the disc diffusion test results, *A. agallocha* roots may have medicinal applications, particularly against E. *faecium, L. monocytogenes* ATCC 7644, *B. subtilis* DSMZ 1971, *C. albicans* DSMZ 1386, *S. epidermidis* DSMZ 20044 and *S. aureus* ATCC 25923.[7]

Haridra Juice also consists of the Krimihara effect. Curcumin was effective against some species and strains: Streptococcus pyogenes (median MIC = 31.25 µg/mL), methicillin-sensitive *S. aureus* (250 µg/mL), *Acinetobacter lwoffii* (250 µg/mL), and individual strains of *Enterococcus faecalis* and *Pseudomonas aeruginosa* (62.5 µg/mL).[8]

Parpata juice extracts and pure compounds were *in-vitro* assessed against seven clinical gram (-) and gram (+) bacteria viz. *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Salmonella typhimurium, Klebsiella pneumonia, Bacillus subtilis, Salmonella typhi.*[9]

CONCLUSION

In vitro, soap showed a significant reduction in CFU, bacteria and fungus immediately, after 5 minutes, after 15 minutes, and after 30 minutes. Also, Nalpamaradi soap reduced pathogens *C. albicans, E. coli, P. aeruginosa,* and *S. aureus* in *in-vitro* analysis. This proves the antimicrobial activities of Nalpamaradi soap.

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