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IN-VITRO ANTIBACTERIAL ACTIVITY OF *ALOE VERA* STEM CRUDE EXTRACT AGAINST PATHOGENIC BACTERIA: *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS PYOGENES*

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Keywords:

Aloe vera, Crude extract, antibacterial activity, Pathogenic bacteria

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ABSTRACT: Infectious diseases caused by bacteria remain a major public health concern, and searching for alternative antimicrobial agents from medicinal plants is essential. *Aloe vera* is a widely used medicinal plant known for its therapeutic properties, including skin care and antimicrobial activity. This study evaluated the antibacterial activity of *Aloe vera* stem crude extracts against *Staphylococcus aureus* (ATCC-25923) and *Streptococcus pyogene*s (ATCC-19615). Crude extracts were prepared by maceration using chloroform, acetone, and ethanol as solvents. Extract yield was determined, and antibacterial activity was assessed using the disk diffusion method at concentrations of 100, 200, and 300 mg/ml. Ciprofloxacin and tetracycline were used as positive controls, while dimethyl sulfoxide (DMSO) served as a negative control. Minimum inhibitory concentrations (MICs) were determined using the serial dilution method. Among the solvents, ethanol produced the highest extract yield of 0.8%. All extracts demonstrated antibacterial activity against the tested pathogens, with the chloroform extract showing the strongest inhibitory effect. Stem chloroform and sptem acetone extract have better minimum inhibitory concentrations (50 mg/ml). These findings suggest that *Aloe vera* stem extracts possess potential antibacterial properties and may serve as a source of alternative antimicrobial agents.

INTRODUCTION: A pathogen, such as a virus, bacteria, parasite, or fungus, causes an infectious disease. Through contact with human fluids, respiratory droplets, or infected objects, these pathogens can pass from person to person [1]. Plants have inherent therapeutic potential for specific infectious diseases; some significant modern medications are developed from ancient medicinal plant [2].

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For their essential medical requirements, more than 80% of the world's population relies on traditional medicine, which is primarily based on plants [3]. Globally, most of the world's population depends on traditional medicine to meet their primary healthcare needs, with medicinal plants serving as the main source of treatment [4].

Some healing herbs can combat human pathogenic germs. The plant extracts of *Vateria indica*, *Combretum latifolium, Persea macrantha*, and *Humboldtia brunonis* in various solvents demonstrated notable effectiveness [5]. In Ethiopia, many plants have effective medicinal values. In the country, people have a long history of using medicinal plants to treat a variety of diseases. Indigenous people by healers orally transferred

most of the traditional knowledge to use medicinal plants. These plants have been used to treat human and livestock ailments in different areas of the country [6-7]. The world has more than 550 species of aloe [8]. There are 46 species of the genus Aloe among them, and found throughout Ethiopia's floristic zones [9]. *Aloe bertemariae, Aloe friisii, Aloe macrocarpa, Aloe percrassa, Aloe harlana, and Aloe clarkei are* some of the aloe species. It is primarily found in the country's central, southwest, and northern regions [10]. *Aloe vera* is one of the most popular aloe species, and it has antibacterial properties against some bacterial strains, including *Enterococcus bovis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Morganella morgana,* and *Klebsiella pneumonia* [11].

Aloe vera gel is a natural substance that has a variety of uses. It can use meals and beverages like aloe juice to treat infections and skin conditions [12-13]. *Aloe vera* plant components have a wide range of potential uses in Ethiopia, but they are not fully utilized since there are no scientific data [14]. The antibacterial activity of *Aloe vera* stem extracts has not been studied sufficiently. The makeup and potency of the *Aloe vera* stem might vary depending on factors like genotype, soil type, environmental factors, harvesting techniques, extraction techniques, and bacterial strain [15].

MATERIAL AND METHODS:

Plant Material Collection and Preparation: The plant *Aloe vera* was collected and authenticated at the Department of Botany, Addis Ababa University, and a voucher specimen was deposited under voucher number EM-001. After authentication, mature fresh stems with a plastic bag were collected from Dilla University Botanical Garden. The fresh stem of the plant was cleaned individually with tap water and then distilled water after being brought into the laboratory to eliminate dirt and soil particles and prevent contamination. The plant samples were carefully and continuously monitored in the botanical lab at Dilla University for six weeks while being cut into little pieces and shade-dried with newspaper on the table at room temperature. To create a suitable fine powder, the dried samples were ground in a sterilized electric grinder and then passed through a filter with a mesh size of 0.5 mm [16]. 200 g of powder was then weighed for each solvent and kept in a closed bottle until use.

Preparation of Plant Extracts and Extraction Yield: A maceration technique was used for crude extraction [17]. The maceration crude extraction method was used to extract from a plant sample (stem) using an analytical grading solvent with chloroform (Sigma Aldrich, Germany), acetone (Sigma Aldrich, Germany), and ethanol (Merck, United Kingdom) as nonpolar- intermediate, less polar and high polar solvents respectively. Crude extraction was done using ethanol, chloroform, and acetone by modifying the maceration process. *Aloe vera* stem powder weighing 200g was finely powdered and then macerated in three different flasks with a 1:5 w/v mixture of ethanol, acetone, and chloroform, respectively [16]. The powder was frequently stirred on an orbital shaker at 180 rpm while being macerated in chloroform, acetone, and ethanol at room temperature for 48 hours. The maceration was filtered using Whatman No. 1 filter paper after complete dissolution (48 hours), yielding filtrate and residue. To obtain crude extracts, the filter was evaporated using Rota vapour at 40 °C under decreased pressure with a vacuum sucker. The product of crude extracts was collected, weighed in grams (g), and kept at 4°C in a refrigerator in bottles with caps and labels. By the formula from Shown Blow, the weight of the yield product and its percentage were computed [18].

Yield of extract (%) = Weight of extract obtained (g) / Weight of plant material used (g) x 100

Antibacterial Activity of the Plant Extract:

Collection and Culturing of the Test Organism: Two bacterial species, namely *Staphylococcus aureus* (ATCC-25923) and *Streptococcus pyogenes* (ATCC-19615) were selected based on their frequent pathogenicity on the skin. These bacteria species that cause serious infections in humans were used for the study. The bacterial sample was collected from an Ethiopian public health institution in Addis Ababa. The bacterial samples were taken in an ice box and brought to the Dilla University microbiology research laboratory. Mannitol salt agar (MSA) and blood agar (111.02g in 1000 ml of distal water and 40g in 950 ml of distilled water with 50 ml of sheep blood) were prepared respectively.

Selected bacterial skin pathogens (*Staphylococcus aureus* and *Streptococcus pyogenes*) were cultured on Mannitol salt agar and blood agar media respectively. The bacterial strains were incubated at 37°C for 24 hours. A few colonies of each strain were transferred with a sterile inoculating loop to a sterile broth nutrient medium and cultured for 24 hours until the turbidity was adjusted to that of 0.5 McFarland turbidity standards. After culture on the nutrient broth media, the bacterial samples were spread and cultured on Muller-Hinton agar by dividing the plate into five parts to test the three concentrations with positive and negative controls of the crude extract. The remaining ones were preserved in the refrigerator for additional use.

Preparation of Test Solution: The standard crude extracts of the stem were diluted with DMSO (10 ml distilled water with 90 ml DMSO) solution to make three different concentrations in a separate beaker with working stock solutions of 100, 200, and 300 mg/ml. The first working solution was prepared by transferring 100 mg of each extract to a sterile test tube containing 1 ml of DMSO solution to get a concentration of 100 mg/ml; the second and third working concentrations were prepared similarly for all extracts 19. 20 µg of ciprofloxacin and 80 µg of tetracycline were dissolved with 1 ml of DMSO solution for *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria, respectively, as positive controls, while 1 ml of DMSO solution was used as a negative control, and the stock solution was stored at 4°C until used.

Antibacterial Activity Test: Six mm diameter disc were prepared from Whatman No. 1 filter paper with a puncher and sterilized using an autoclave [19]. The culture of the test isolated bacteria was streaked on an independent surface of prepared MHA plates by using a sterile cotton swab that was used to apply the suspension uniformly. 30 µL of 100 mg/ml, 200 mg/ml, and 300 mg/ml concentrations of solution were applied to a sterilized disc using a micropipette. After being completely absorbed and slightly dried, the disc was placed on prepared MHA culture media with the use of sterilized forceps. 20 µg of ciprofloxacin and 80 µg of tetracycline were dissolved in 1 ml of DMSO solution, and 30 µL were applied to a sterilized disc to use as a positive control from the prepared solution. 30 µL of DMSO solution was applied to the disc to use as a negative control. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed, and the diameter (mm) was measured to assess bacterial growth and the effect of a specific concentration of plant extract. The test was repeated three times, and the average zone of inhibition diameter was recorded.

Determination of Minimum Inhibitory concentration (MIC): Bifold dilution was used to determine the minimum inhibitory concentrations (MIC) of chloroform, acetone, and ethanol crude extracts that were tested against isolated bacteria [20]. Different concentrations of the extracts (200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml) were dispensed into each six mm-diameter sterile disc, and each different concentration of the disc was labeled. Each disc's concentrations were put on MHA-prepared culture media.

The prepared cultures were incubated at 37°C for 24 hours. The zones of inhibition were observed and recorded. The lowest concentration of the agent that prevents the growth of the bacteria was taken as the MIC, and the entire test was made in triplicate.

Data Analysis and Interpretation: All the data obtained from the experimental results were recorded by measuring the zone of inhibition (in mm) with its control and the crude extract of each bacterium. The data were taken as the average value (mean ± standard deviation of the mean value of the test).

The result was a one-way ANOVA performed to test the variation of mean concentrations with control and among them of the crude extract. A Tukey post hoc. The test was used to compare the association between the zone of inhibition among concentration groups with positive and negative controls. P-values < 0.05 were considered statistically significant differences.

RESULTS AND DISCUSSION:

Percent of Yield and Crude Mass of Extract: Using various solvents, such as chloroform, acetone and ethanol, crude extracts of Aloe vera stems showed a range of values for their crude mass and percent yields of the extract. The results of the matching crude mass and yield percentage

were detailed and shown in **Table 1**. The yield and crude mass of the stem extracts in chloroform, acetone and ethanol were 1.2 g (0.6%), 1.5 g (0.75%), and 1.6 g (0.8%), respectively.

TABLE 1: WEIGHT AND PERCENTAGE YIELD OF STEM CRUDE EXTRACT OF ALOE VERA PLANT USING CHLOROFORM, ACETONE AND ETHANOL SOLVENT FROM 200G OF DRY POWDER

Plant part	Solvent type	Weight of dry powder (g)	Weight of crude extract(g)	Yield (%)	Extract color
Stem	Chloroform	200g	1.2	0.6%	Green dark
	Acetone	200g	1.5	0.75%	Green dark
	Ethanol	200g	1.6	0.8%	Green dark

Based on the crude mass collected, ethanol has the highest extract yield, followed by acetone and chloroform **Table 1.** This outcome is consistent with the results obtained with ethanol, acetone and chloroform, with ethanol obtaining the highest yield extract [18]. This implies that polarity has a major impact on the yield of crude extracts from plant sources [21].

Antibacterial Activity of the Stem Extract: Stem chloroform, acetone and ethanol crude extracts showed antibacterial activity on both bacterial strains (*Staphylococcus aureus* and *Streptococcus pyogenes)* with different antibacterial effects. Ciprofloxacin and tetracycline were used as positive controls on *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria respectively **Fig. 1** & **Table 2.**

The concentration levels of 100 mg/ml, 200 mg/ml and 300 mg/ml of stem chloroform extract had antibacterial activity. The inhibitory mean diameter values were 12.83±0.29, 13.33±0.58 and 16.50±0.50 mm, respectively, and a positive control had 15.17±0.29 mm on *Staphylococcus aureus* **Table 2.** The three concentration levels had a significant difference as compared to the positive control with a p-value of 0.00, and they had a significant difference as compared to the negative control with a p-value of 0.00. This indicates that the crude extract of stem chloroform extract had a lower antibacterial activity as compared to the positive control and had a better antibacterial activity as compared to the negative control on *Staphylococcus aureus* bacteria. Similarly, stem chloroform extract had an antibacterial activity on *Streptococcus pyogenes* bacteria. The three concentrations levels of stem chloroform extract, 100 mg/ml, 200 mg/ml, and 300 mg/ml, have mean values of 11.33±0.58 and 15.67±0.58 mm, respectively, with a 16.17±0.29 mm mean of positive control **Table 2.**

The concentrations of 100 mg/ml and 200 mg/ml have a significant difference as compared to the positive control, with a p-value of 0.00. The concentrations of 100 mg/ml and 200 mg/ml also have a significant difference as compared to the negative control, with a p-value of 0.00. This indicates the two concentrations had a lower antibacterial effect as compared to the positive control and a better antibacterial effect as compared to the negative control. However, 300 mg/ml is an insignificant difference as compared to the positive control with a p-value of 0.56. This implies that the positive control and 300 mg/mL showed similar antibacterial activity.

Stem acetone extract has antibacterial activity on *Staphylococcus aureus.* The three concentrations of stem acetone extract were 100 mg/ml, 200 mg/ml and 300 mg/ml, with a mean value of 9.00±0.00 mm, 10.17±0.29 mm and 12.77±0.25 mm, respectively, and a positive control has a mean value of 15.17±0.29 mm **Table 2.** The three concentrations of the stem acetone extract and the positive control have a significant difference with a p-value of 0.00. The three concentrations also have a significant difference as compared to the negative control, with a p-value of 0.00. As indicated by the mean value of the three concentrations and the positive control with a p-value in **Table 2**.

The three concentrations have less antibacterial activity as compared to the positive control, and these concentrations have better antibacterial activity as compared to the negative control. The three concentrations also have a significant difference between each other with a p-value of 0.00 for *Staphylococcus aureus* bacteria. Likewise, stem acetone extract had an antibacterial activity on *Streptococcus pyogenes* bacteria. The three concentrations of 100 mg/ml, 200 mg/ml, and 300 mg/ml have mean values of 9.00±0.00, 10.17±0.29, and 12.77±0.25 mm, respectively, and the positive

control has a mean value of 15.17±0.29 mm. The three concentrations and the positive control have a significant difference with a p-value of 0.00, and their concentrations have a significant difference as compared to the negative control with a p-value of 0.00. This implies that the three concentrations have lower antibacterial activity than the positive control and a better antibacterial activity than the negative control. The three concentrations also have significant differences from each other, with a p-value of 0.00 for *Streptococcus pyogenes* bacteria. It indicated that the antibacterial activity increases with the concentration of the crude extract.

Stem ethanol extract has antibacterial activity on *Staphylococcus aureus.* The concentrations of stem ethanol extract were 100 mg/ml, 200 mg/ml, and 300 mg/ml, with mean values of 7.33±0.58, 10.33±0.58, and 11.67±0.58 mm, respectively, and a positive control had a mean value of 15.00±0.00 mm **Table 2**. The three concentrations have a significant difference as compared to the positive control, with a p-value of 0.00. The three concentrations also have a significant difference as compared to the negative control, with a p-value of 0.00. This implies that the crude extract of stem ethanol extract has lower antibacterial activity as compared to the positive control, and the three concentrations have better antibacterial activity as compared to the negative control. The concentrations also have a significant difference between each other with a p-value of 0.00 on *Staphylococcus aureus*. This describes antibacterial activity that increases with increasing concentration. In the same way, stem ethanol crude extract has an antibacterial effect on *Streptococcus pyogenes* bacteria. The three concentrations of 100 mg/ml, 200 mg/ml, and 300 mg/ml have mean values of 0.00±0.00, 7.67±0.58, and 11.17±0.76 mm, respectively, and the positive control mean was 15.83±0.29 mm **Table 2.** The three concentrations of 100 mg/ml, 200 mg/ml and 300 mg/ml have significant differences as compared to the positive control with a p-value of 0.00, and their concentrations have significant differences as compared to the negative control with a p-value of 0.00. The three concentrations had less antibacterial activity as compared to the positive control and better antibacterial activity as compared to the negative control.

Antibacterial activity of *Aloe vera* stem chloroform, acetone and ethanol extract was evaluated against *Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strain and its extract have antibacterial effect with concentration increase antibacterial activity also increase.

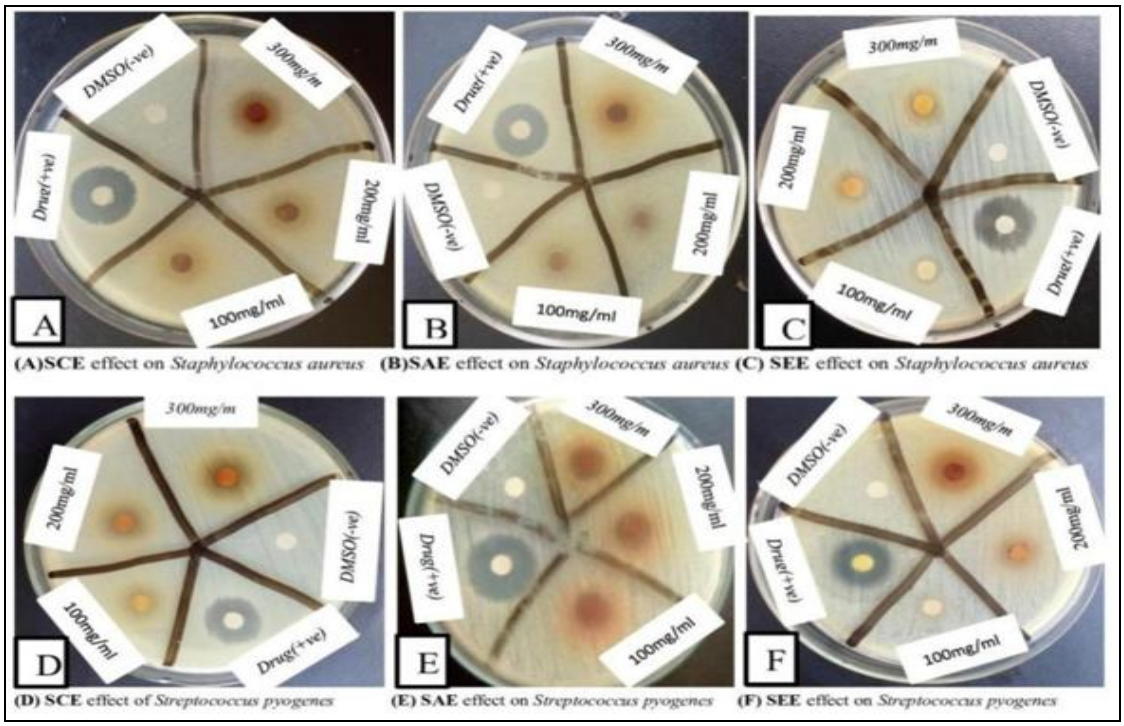

FIG. 1: ZONE OF INHIBITORY BY STEM CHLOROFORM, ACETONE AND ETHANOL CRUDE EXTRACT ON *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS PYOGENE*

TABLE 2: SUMMARY OF INHIBITORY ACTIVITY WITH MEAN±SD AND P-VALUE OF STEM CHLOROFORM, ACETONE AND ETHANOL CRUDE EXTRACT (IN MM)

Test material	Concentration	*Staphylococcus aureus*		Concentration	*Streptococcus pyogenes*	
		Mean ± SD	P-value		Mean ± SD	P-value
Drug(+ve)	20µg/ml	15.17±0.29		80µg/ml	16.17±0.29	
	100mg/ml	12.83±0.29	0.00	100mg/ml	11.33±0.58	0.00
SCE	200mg/ml	13.33±0.58	0.00	200mg/ml	12.83±0.29	0.00
	300mg/ml	16.50± 0.50	0.02	300mg/ml	15.67±0.58	0.56
Drug(+ve)	20µg/ml	15.17 ±0.29		80µg/ml	16.17±0.29	
	100mg/ml	9.00±0.00	0.00	100mg/ml	10.67±0.29	0.00
SAE	200mg/ml	10.17±0.29	0.00	200mg/ml	12.17±0.29	0.00
	300mg/ml	12.77±0.25	0.00	300mg/ml	13.83±0.29	0.00
Drug(+ve)	20µg/ml	15.00±0.00		80µg/ml	15.83±0.29	
	100mg/ml	7.33±0.58	0.00	100mg/ml	0.00±0.00	0.00
SEE	200mg/ml	10.33±0.58	0.00	200mg/ml	7.67±0.58	0.00
	300mg/ml	11.67±0.58	0.00	300mg/ml	11.17±0.76	0.00

SCE = stem chloroform extract, SAE = stem acetone extract, SEE = stem ethanol extract

The result of this investigation was consistent with previous findings of *Aloe vera* extract. The antibacterial activity of ethanol, chloroform, and acetone extracts was evaluated against *Staphylococcus aureus* and Streptococcus pyogenes. The chloroform extract produced the largest zones of inhibition, 15–25 mm, the ethanol extract had an inhibition of 12–20 mm, and the acetone extract showed an inhibition of 10–18 mm against both bacterial strains [22]. Variation in the zone of inhibition of extracts may occur due to differences in extraction techniques and plant parts, as leaves and stems contain varying amounts of bioactive compounds [23].

Minimum Inhibitory Concentration (MIC): The disc diffusion method was used because it is simple, cost-effective, and suitable for preliminary antimicrobial screening. Discs containing serially decreasing extract concentrations were applied, and the lowest concentration producing a visible inhibition zone was considered an approximate MIC. Minimum inhibition concentrations were done starting from two hundred due to the common minimum inhibitory concentration for all solvent extracts on both bacterial strains. In this test, SCE, SAE and SEE showed minimum inhibitory concentrations on *Staphylococcus aureus* and *Streptococcus pyogenes*; see **Table 3**. The MIC of SCE and SAE was 50 mg/ml on both tested bacteria, and SEE has MICs of 100 mg/ml and 200 mg/ml for *Staphylococcus aureus* and *Streptococcus pyogenes*, respectively. The plus sign indicates that they have an antibacterial effect, and the minimum concentration has a zone of inhibition of 7-9 mm for each extract and bacterial strain.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION OF STEM WITH CHLOROFORM, ACETONE AND ETHANOL SOLVENT CRUDE EXTRACT AGAINST TESTED BACTERIA

Plant extract type	Concentration	*Staphylococcus aureus*	*Streptococcus pyogenes*
	200mg/ml	+++	+++
	100mg/ml	++	++
	50mg/ml	+	+
SCE	25mg/ml	-	-
	12.5mg/ml	-	-
	6.25mg/ml	-	-
	3.125mg/ml	-	-
	200mg/ml	+++	+++
	100mg/ml	++	++
	50mg/ml	+	+
SAE	25mg/ml	-	-
	12.5mg/ml	-	-
	6.25mg/ml	-	-
	3.125mg/ml	-	-
	200mg/ml	++	+
	100mg/ml	+	-

SEE	50mg/ml	-	-
	25mg/ml	-	-
	12.5mg/ml	-	-
	6.25mg/ml	-	-
	3.125mg/ml	-	-

SCE = stem chloroform extract, SAE = stem acetone extract, SEE = stem ethanol extract, - = No effect, + = weak effect, ++ = moderate effect, +++ = strong effect, ++++ = very strong effect

CONCLUSION: Among the crude extracts obtained, ethanol produced the highest extraction yield, followed by acetone and chloroform. All extracts exhibited antibacterial activity against both tested bacteria, with the chloroform stem extract showing the strongest effect. The minimum inhibitory concentration (MIC) of each extract was also determined. These findings indicate that *Aloe vera* stem extracts have potential as a natural source of antibacterial agents for the treatment of skin infections; however, further studies are required to confirm their efficacy and elucidate their mechanisms of action.

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CONFLICTS OF INTEREST: Nil

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