

## Research article

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# The Characteristics of Wood Vinegar from Cocoa Pod Husks as an Antifungal Agent for Natural Rubber Sheets

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## Abstract

Natural rubber sheets are susceptible to fungal contamination during storage, leading to quality degradation and economic losses. Traditional antifungal treatments often involve synthetic chemicals, which raise environmental and health concerns. Wood vinegar, a byproduct of biomass pyrolysis, has emerged as a potential natural antifungal agent. The aims of this study were to evaluate the physical and chemical properties as well as the antifungal activity of wood vinegar derived from cocoa pod husks, and comparing it with wood vinegar from rubberwood and bamboo. Wood vinegar was produced through pyrolysis at 400°C, and its components were qualitatively identified using gas chromatography–mass spectrometry (GC/MS). Antifungal activity was assessed at concentrations ranging from 15% to 60% using the spread plate method to evaluate fungal inhibition. The results showed that wood vinegar from cocoa pod husks exhibited medium quality in terms of odor, color, and transparency. It had a pH of 4.8, a total soluble tar content of 0.12% (wt), a specific gravity of 1.003 g/mL, and a °Brix of 1.5. The major components of cocoa pod husk wood vinegar were acetic acid (36.90%) and phenol (4.61%), with concentrations close to those found in bamboo wood vinegar. Wood vinegar from cocoa pod husks demonstrated inhibitory effects on fungal growth, significantly reducing fungal counts on rubber sheets. Higher concentrations of wood vinegar correlated with greater fungal inhibition, with a 60% concentration reducing fungal growth to  $5.6 \times 10^4$  CFU/g, while the control rubber sheet had  $1.1 \times 10^6$  CFU/g during storage. However, at the same concentration, wood vinegar derived from rubberwood exhibited the highest antifungal efficacy, followed by bamboo, while cocoa pod husk wood vinegar showed the least effectiveness among the three.

**Keywords:** cocoa pod husks; wood vinegar; antifungal; natural rubber sheets

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## 1. Introduction

Cocoa cultivation in Thailand is expanding rapidly. In 2022, the country produced 1,256 tons of cocoa, accounting for just 0.02% of global production. By 2023, however, production had surged to 3,360 tons, reflecting a substantial increase in both cultivation and processing activities (Thailand Breaking News, 2024). This growth has resulted in a significant increase in cocoa pod husks (CPHs)—the fibrous outer shells of cocoa beans. Traditionally treated as waste, CPHs are rich in cellulose (20.15%), hemicellulose (21.06%), and lignin (51.98%) (Wijaya et al., 2017), making them a valuable source of lignocellulosic biomass. The sustainable utilization of agricultural waste is increasingly recognized as crucial for the development of bio-based alternatives to chemical agents. In this context, the production of wood vinegar presents a sustainable solution by transforming cocoa pod husks into a valuable resource.

Wood vinegar is a liquid product, typically brown or reddish-yellow, derived from wood or wood residues, including those from the wood processing industry, tree branches, rubberwood, bamboo, crop straw, fruit shells, and other biomaterials (Wei et al., 2010). It is produced through the thermal decomposition (pyrolysis) of these materials in the absence of oxygen or under a controlled atmosphere (Pimenta et al., 2018). This process breaks down the organic components in biomass, releasing volatile compounds such as acetic acid, methanol, and various phenols, which contribute to its characteristic smoky odor and complex chemical composition. Wood vinegar has gained attention for its potential benefits in both agricultural and industrial applications. It contains natural compounds with antimicrobial and antifungal properties.

Fungal contamination of natural rubber sheets is a persistent challenge in the rubber industry, particularly in humid tropical regions. Rubber sheets, essential to various rubber products, are highly susceptible to colonization by fungal species such as *Aspergillus*, *Penicillium*, and *Paecilomyces* (Roy & Dutta, 2003). Such contamination can lead to discoloration, unpleasant odors, and compromised mechanical properties, reducing the overall quality of raw rubber. Wood vinegar has shown promise as a natural antifungal agent in rubber processing, with efficacy linked to its phenolic compound content (Baimark & Niamsa, 2009). However, comparative studies evaluating the antifungal effectiveness of wood vinegar from different biomass sources are limited.

The present study aimed to evaluate the chemical composition and antifungal efficacy of wood vinegar produced from cocoa pod husks. The wood vinegar was characterized using gas chromatography–mass spectrometry (GC-MS) and compared with that derived from rubberwood and bamboo. Furthermore, its antifungal activity was assessed across a range of concentrations (15% to 60%) on natural rubber sheets. The findings provide insight into the potential of CPH-derived wood vinegar as a sustainable antifungal agent, offering an innovative solution for fungal control in the rubber industry while advancing the circular utilization of agricultural waste.

## 2. Materials and Methods

### 2.1 Preparation of wood vinegar

Cocoa pod husks, rubberwood and bamboo were sun-dried for five days. After drying, 40 kg of each material were placed into a 200-L horizontal kiln and pyrolyzed at 380°C for 8 h. The smoke generated from thermal decomposition was passed through a tar separation

column and condensed using a condenser. The wood vinegar liquid was collected in a 500 mL opaque container (Chungsiriporn et al., 2020).

## **2.2 Analysis of physical properties**

The pH of the wood vinegar was measured using a pH meter (Lutron PH-222), while the specific gravity was determined using a pycnometer method. The odor and color were also observed. Odor was assessed through direct sensory evaluation, noting characteristics such as smokiness or pungency. Color was evaluated visually under standardized lighting conditions. The total soluble tar content was evaluated by transferring 0.5 g of wood vinegar to a calibrated vial and heating it at 105°C to remove any volatile components. The remaining residue was used to calculate the tar content. Brix was measured using a hand refractometer (Duralab, Brix range: 0-32%) without dilution (Nibalvos et al., 2021). Each treatment was repeated three times.

## **2.3 Analysis of the chemical composition of wood vinegar by GC–MS**

GC-MS analysis was performed using the Shimadzu GC-MS-QP 2010 Ultra instrument, equipped with an Rtx-1MS stationary phase. The carrier gas, ultra-high purity helium, was set to a flow rate of 1 mL/min with a 10:1 split ratio, and the injection volume was 2 µL. The column temperature was initially set to 110°C for 5 min, with a ramp rate of 10°C/min. The temperature was then increased to 200°C and held for 5 min, followed by a further increase to 250°C at a rate of 5°C/min, with a final hold of 5 min. The injector temperature was maintained at 250°C throughout the analysis (Kumar et al., 2008). The components were identified using area%.

## **2.4 Testing of wood vinegar on rubber sheets**

The rubber sheets used in this study were obtained from the Songkhla Rubber Regulatory Center, Songkhla, Thailand. Each rubber sheet was cut into square samples measuring 12 cm × 12 cm. Wood vinegar derived from cocoa pod husks was diluted with distilled water to prepare solutions at concentrations of 15%, 30%, 45% and 60% (v/v), 30% was used for rubberwood and bamboo to compare with the same mid-level concentration of cocoa pod husk wood vinegar. Each rubber sheet sample was individually immersed in 200 mL of the prepared wood vinegar solution for 10 min. After that, the rubber sheets were removed from the solution, soaked, air-dried at room temperature and stored in a controlled environment using a potassium sulfate solution to maintain a relative humidity of 96±1% for six days. Each treatment was repeated three times.

## **2.5 Analysis of fungal growth by spread plate method**

The growth of fungi on the surface of the rubber sheets was measured to evaluate antifungal activity. Colony counting was performed using the spread plate method, as described by Tournas *et al.* (2001). Each rubber sheet sample, measuring 2 cm × 2 cm, was stacked in four layers. A 100 mL solution of 0.1% peptone was added to the stacked sheets and shaken for 30 min to prepare the sample solution. The sample solution was then pipetted onto the surface of Potato Dextrose Agar (PDA), with 0.1 mL applied per plate. The solution was spread over the agar surface and allowed to permeate for approximately 10 min. Agar plates were then incubated at 20-25°C for five days (three

replicates were performed). After incubation, the number of colonies was counted and expressed as the decrease in the number of colonies per gram (CFU/g).

## 2.6 Statistical analysis

Statistical analysis was carried out by one-way ANOVA, and Duncan's new multiple range test was employed to distinguish means and differences. The statistical analysis was considered significant at  $p < 0.05$ , utilizing SPSS version 12.0.

## 3. Results and Discussion

### 3.1 Physical characteristics of wood vinegar

The physical characteristics of wood vinegar derived from cocoa pod husks, rubberwood and bamboo, which were pyrolysis under the same conditions, are presented in Table 1. The results indicate that wood vinegar from cocoa pod husks was acidic with a pH of 4.8, a total soluble tar content of 0.12% (wt), a specific gravity of 1.003 g/mL and a °Brix value of 1.5. Typically, the acidity of wood vinegar falls within a pH range of 2-4 because of the presence of acetic acid, formic acid, and propionic acid (Ouattara et al., 2023). Reported values for total soluble tar content ranged from 0.23% to 0.89% wt, while specific gravity and °Brix values ranged from 1.005 to 1.016 g/mL and 1.7 to 6.6, respectively (Yoshimoto, 1994). The acidity of wood vinegar significantly influences the solubility of allelochemical compounds, such as organic acids and phenols, thereby enhancing their effects under acidic conditions (Armstrong & Armstrong, 1999). Based on the statistical results, the physical properties of wood vinegar from cocoa pod husks differed notably from the other two sources, potentially influencing its biological effectiveness.

**Table 1.** Physical characteristics of wood vinegar derived from cocoa pod husks, rubberwood and bamboo

Sample	Physical Parameter*				
	Color	pH	SG (g/mL)	°Brix	Total Soluble Tar (%by weight)
Cocoa pod husk	pale yellow	4.80±0.12 <sup>c</sup>	1.003±0.05 <sup>b</sup>	1.50±0.10 <sup>c</sup>	0.12±0.02 <sup>a</sup>
Rubberwood	reddish	3.69±0.10 <sup>a</sup>	1.012±0.03 <sup>a</sup>	5.00±0.11 <sup>a</sup>	0.34±0.01 <sup>b</sup>
Bamboo	reddish	3.96±0.09 <sup>b</sup>	1.010±0.03 <sup>a</sup>	4.00±0.11 <sup>b</sup>	0.52±0.04 <sup>c</sup>

\*Values are mean±standard deviation (n=3), while difference letters for values in each column indicate significant differences ( $p < 0.05$ ).

All wood vinegar solutions had varying colors, with wood vinegar from cocoa pod husks appearing pale yellow, whereas those from bamboo and rubberwood exhibited a reddish hue. The pH of wood vinegar derived from rubberwood and bamboo was lower than that of vinegar obtained from cocoa pod husks. Total soluble tar content was also significantly higher in rubberwood (0.34%) and bamboo (0.52%) compared to cocoa pod

husks (0.12%), indicating a richer concentration of non-volatile components. The specific gravity values for rubberwood (1.012) and bamboo (1.010) fell within Japan's quality assessment standards (1.010-1.050) (Wada, 1997) and were significantly higher ( $p < 0.05$ ) than that of cocoa pod husk vinegar (1.003), which fell below the standard. The °Brix values of wood vinegar from rubberwood and bamboo ranged from 4.00 to 5.00 were also significantly higher than that of cocoa pod husks (1.5), aligning with the recommended range of 1.7-6.6 (Mun et al., 2007). °Brix values may serve as a general indicator of tar content in wood vinegar (Theapparatt et al., 2014).

### 3.2 Chemical component of wood vinegar

The chemical composition of wood vinegar obtained from the pyrolysis of cocoa pod husks, rubberwood, and bamboo revealed the predominance of 10 compounds: acetic acid, phenol, 2,6-dimethoxyphenol, 2-propanone, 1-hydroxy-3,5-dimethoxy-4-hydroxytoluene, 2-methoxyphenol, 4-ethyl-2,6-dimethoxyphenol, propanoic acid, 2-cyclopenten-1-one, 2-hydroxy-3-methyl- and creosol, which were found in significantly higher amounts than the other compounds, as presented in Table 2. The results indicate that acetic acid was the predominant component in all samples. Among the various chemical constituents of wood vinegar, acetic acid is recognized as the primary compound responsible for its biological activities, including antifungal properties and other functional applications.

**Table 2.** Component of wood vinegar from cocoa pod husks, rubberwood and bamboo

No.	Retention Time	Component	Area%		
			Cocoa Pod Husks	Rubberwood	Bamboo
1	10.9746	2-Propanone, 1-hydroxy	4.51	3.96	4.07
2	15.2510	Acetic acid	36.90	45.28	37.58
3	17.5754	Propanoic acid	2.33	2.16	2.92
4	24.1988	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	1.63	2.05	1.94
5	24.8069	Phenol, 2-methoxy-	3.20	2.72	2.79
6	26.7931	Creosol	1.31	1.32	1.08
7	27.8246	Phenol	4.61	1.33	5.55
8	24.0730	Phenol, 2,6-dimethoxy-	9.16	5.78	4.93
9	35.4113	3,5- Dimethoxy-4-hydroxytoluene	2.70	2.31	1.62
10	36.1785	4-Ethyl-2,6-dimethoxyphenol	2.62	1.52	0.97

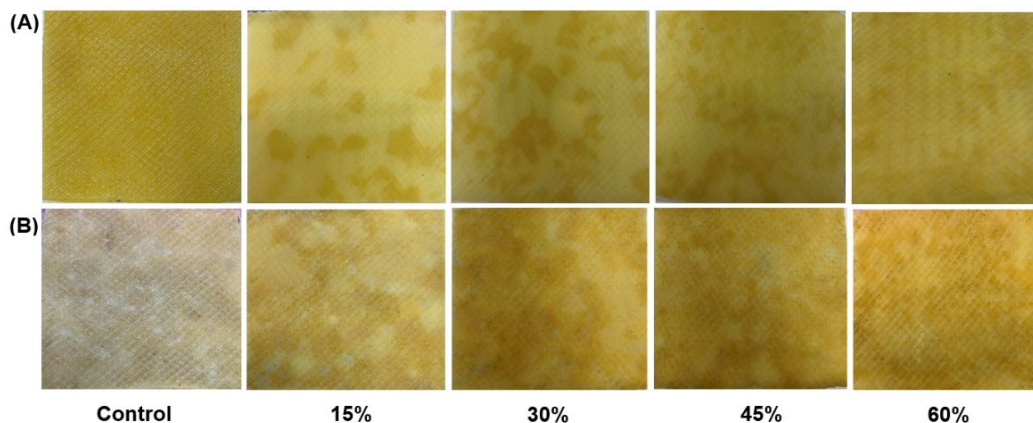
Among the main components analyzed, wood vinegar from rubberwood had the highest acetic acid content (45.28%) and phenol content (1.33%), which may enhance its antifungal, antimicrobial, and preservative qualities due to the elevated levels of these

compounds. In comparison, wood vinegar from cocoa pod husks contained 36.90% acetic acid and 4.61% phenol, values similar to those of bamboo vinegar. Phenol and acetic acid are major constituents of wood vinegar and contribute significantly to its overall quality (Prianto et al., 2020). The phenolic and acid concentrations in wood vinegar were very important for its antifungal effect (Yahayu et al., 2017). Acetic acid, the most abundant component in wood vinegar, is believed to originate from the acetyl groups in hemicellulose (Kartal et al., 2004). Acetic acid and creosol contribute to the characteristic odors of wood vinegar, including sour, smoky and medicinal notes (Akakabe et al., 2006).

In addition to acetic acid, phenolic compounds such as creosol, 2,6-dimethoxyphenol and others play a pivotal role in the unique properties of wood vinegar. These phenols, including 2,6-dimethoxyphenol, which were found at higher concentrations in cocoa pod husks, have shown to possess potent antioxidant, antimicrobial, and antifungal properties (Prianto et al., 2020). The higher phenolic content in cocoa pod husk wood vinegar is likely attributed to the relatively high lignin content in cocoa pods (ranging from 14% to 38.8%), which is a known source of phenolic compounds upon thermal degradation (Younes et al., 2023). This suggests that wood vinegar derived from cocoa pod husks can provide protection against fungal contamination.

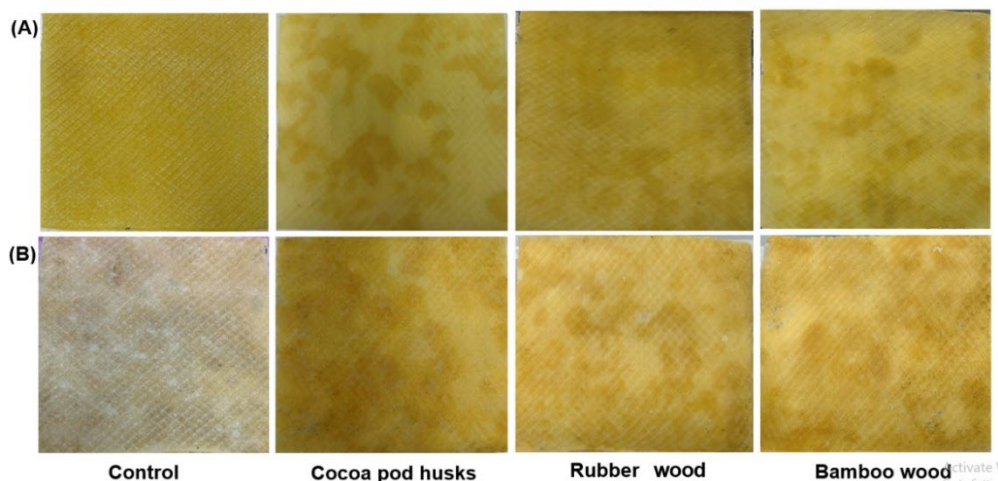
### 3.3 Antifungal performance of the natural rubber sheets

Surface images of the natural rubber sheets treated with cocoa pod husks wood vinegar at various concentrations are presented in Figure 1. The results showed that after storage, the control rubber sheet had faded in color, with white and black spots likely caused by fungal or bacterial growth on its surface. In contrast, rubber sheets treated with wood vinegar at concentrations ranging from 15% to 60% exhibited reduced fungal growth and retained their original color. This indicates that cocoa pod husks wood vinegar, even at lower concentrations, possesses antifungal properties that effectively protect rubber surfaces from fungal contamination.



**Figure 1.** Effect of wood vinegar derived from cocoa pod husks at concentrations of 15, 30, 45 and 60% v/v on rubber sheets: (A) before storage and (B) after storage

The antifungal performance of natural rubber sheets treated with wood vinegar derived from different sources—cocoa pod husks, rubberwood and bamboo—at a 30% concentration is photograph presented in Figure 2. The results indicate that all three types of wood vinegar preserved the original color of the rubber sheets after storage, suggesting a protective effect against environmental factors that promote fungal contamination. However, the extent of antifungal activity varied among the different wood sources. Wood vinegar derived from rubberwood exhibited the highest antifungal effectiveness, as demonstrated by the minimal occurrence of white and black fungal spots on the rubber sheets. This is likely due to its higher concentration of bioactive phenolic and acidic compounds, which have been reported to inhibit fungal growth (Kalasee & Teekapakvisit, 2020; Nun-Anan et al., 2021). In comparison, bamboo vinegar demonstrated moderate antifungal activity, suggesting that while it possesses antifungal properties, its efficacy is slightly lower than that of rubberwood vinegar. Wood vinegar derived from cocoa pod husks showed the least antifungal effectiveness among the three treatments, implying a lower concentration or potency of antifungal compounds. Despite this, its ability to maintain the color of the rubber sheets indicates a certain level of protective effect.



**Figure 2.** Effect of wood vinegar derived from cocoa pod husks, rubberwood and bamboo at a concentration of 30% v/v on rubber sheets: (A) before storage and (B) after storage

The initial rubber sheets had a fungal count of  $1.1 \times 10^3$  CFU/g. The antifungal activity on the rubber sheets increased with higher wood vinegar concentrations. Wood vinegar derived from cocoa pod husks at a 60% concentration significantly reduced fungal growth, resulting in a fungal count of  $5.6 \times 10^4$  CFU/g, compared to the control group, which had a fungal count of  $1.1 \times 10^5$  CFU/g ( $p < 0.05$ ), indicating the antifungal potential of cocoa pod husk-derived wood vinegar. These findings were consistent with those of Desvita et al. (2022), who reported similar antifungal effects. In comparison, wood vinegar from rubberwood at a 30% concentration exhibited a fungal count of  $9.0 \times 10^4$  CFU/g, which was statistically lower than that of bamboo-derived wood vinegar ( $1.2 \times 10^5$  CFU/g) and cocoa pod husk vinegar at the same concentration ( $2.6 \times 10^5$  CFU/g). According to the results presented in Table 3, these differences were also statistically significant ( $p < 0.05$ ). This suggests that, among the materials tested, rubberwood-derived wood vinegar demonstrated the most effective antifungal activity at the same concentration level.

**Table 3.** Fungal count on initial rubber sheets and after storage

Samples	Fungal Count (CFU/g)*
Initial	$1.1 \times 10^3 \pm 0.07^a$
Control	$1.1 \times 10^6 \pm 0.12^g$
15% v/v cocoa pod husks	$5.8 \times 10^5 \pm 0.10^f$
30% v/v cocoa pod husks	$2.6 \times 10^5 \pm 0.07^e$
45% v/v cocoa pod husks	$1.0 \times 10^5 \pm 0.11^d$
60% v/v cocoa pod husks	$5.6 \times 10^4 \pm 0.09^b$
30% v/v rubberwood	$9.0 \times 10^4 \pm 0.13^c$
30% v/v bamboo	$1.2 \times 10^5 \pm 0.08^d$

\*Values are mean±standard deviation (n=3), while difference letters for values indicate significant differences (p<0.05).

The variation in antifungal efficacy between different types of wood vinegar suggests that the chemical composition, influenced by the source material, plays a crucial role in determining the degree of antifungal activity. Cocoa pod husks, which have relatively high phenolic content due to their lignin composition (Younes et al., 2023), appear to contribute significantly to the antifungal properties of the resulting vinegar. In contrast, rubberwood and bamboo, with their distinct sets of bioactive compounds, appear to outperform cocoa pod husks, likely due to synergistic effects between acetic acid and other bioactive components. Literature reports indicate that phenolic compounds and acetic acid in wood vinegar play a significant role in its antifungal properties (Velmurugan et al., 2009; Faisal et al., 2019).

Although cocoa pod husk-derived wood vinegar can significantly reduce fungal growth on rubber sheets at higher concentrations than rubberwood and bamboo, it can still be considered a promising alternative to chemical fungicides. By tapping into agricultural waste, such as cocoa pod husks, this research contributes to the broader movement toward sustainable practices and the circular economy. The application of this natural wood vinegar can help reduce reliance on synthetic chemicals, promote eco-friendly alternatives, and support the development of bio-based products in various sectors. Furthermore, the study provides a foundation for future research to optimize the production and applications of wood vinegar in different fields, including pest control, plant growth regulation, and industrial use.

## 4. Conclusions

The physical and chemical characteristics of wood vinegar derived from cocoa pod husks suggest its potential as an antifungal agent, though its effectiveness appears to be moderate compared to wood vinegar from rubber wood and bamboo. The physical

properties of cocoa pod husk wood vinegar, including a pH of 4.8, a total soluble tar content of 0.12% (wt), a specific gravity of 1.003 g/mL, and a °Brix value of 1.5, fall within an acceptable range for antifungal applications. The chemical analysis of this kind of wood vinegar revealed that acetic acid is the predominant compound. Among the different sources, rubberwood vinegar exhibited the highest acetic acid content (45.28%), while cocoa pod husk vinegar contained 36.90% acetic acid. Overall, acetic acid serves as the primary component responsible for the antimicrobial potential of wood vinegar. Cocoa pod husk wood vinegar at a 30% concentration exhibits lower effectiveness compared to rubberwood and bamboo-derived vinegars. While cocoa pod husk wood vinegar offers a sustainable and value-added use of agricultural waste, its application as an antifungal agent in the rubber industry requires further optimization to match the effectiveness of wood vinegars from other sources.

## 5. Acknowledgements


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## 6. Authors' Contributions

Juntima Chungsiriporn designed the research and wrote the paper; Arisara Romyen coordinated the research; Prukaya Pongyeela analyzed data; and Nirana Chairerk performed the research.

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## 7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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