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Nutritional Composition, Phytochemical Performance, Total Content of Polyphenols, Antioxidant Capacity, and Bioactive Compounds of Yvapurú Fruits (*Plinia cauliflora*)

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ABSTRACT

Background: Autochthonous fruits such as Yvapurú (*Plinia cauliflora*) have beneficial health properties through their bioactive compounds and antioxidant capacity. The goal of this study was to evaluate the nutritional composition, the phytochemical performance, the total content of polyphenols, the antioxidant capacity and bioactive compounds in the Yvapurú´s fruits.

Methods: The macronutrients were analyzed by the Antrona method for carbohydrates, the Kjeldahl method for proteins and the Softlex method for total fat. Phytochemical screening was performed to detect phenols, flavonoids, saponins, alkaloids, steroids, triterpenoids, leucoanthocyanidins and quinones. The polyphenols total content was obtained through the Folin-Ciocalteu method. Antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) method and the identification of bioactive compounds was carried out through Ultra Performance Liquid Chromatography coupled with hybrid triple quadrupole linear ion trap tandem mass spectrometry (UPLC-ESI-QqQLIT-MS/MS) method.

Results: The macronutrients were found in proportions of 47.8%, 7.24% and 1.28% for carbohydrates, proteins and fats, respectively. Phytochemical screening revealed the presence of phenols and triperthenoids. The total polyphenol content was 24 mg/g. The antioxidant capacity was (10,200±334) µmol/100 g.

Conclusion: These findings confirmed that the fruits of *P. cauliflora* are a good source of antioxidants and bioactive compounds that are beneficial for human health both for the prevention and treatment of diseases.

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Introduction

The regular consumption of fruits is widely recommended in dietary guidelines worldwide, because of their richness in nutrients. Furthermore, several studies showed that this consumption can prevent various diseases and disorders due to the presence of bioactive compounds with antioxidant properties (1-3). Despite the abundance of fruits in the world, there is a large number of underexploited native and exotic species, which may be a possible future source of income for the local population, and an opportunity to gain access to special markets, where consumers have interest in the presence of compounds potentially capable of preventing diseases (4, 5).

In parallel, diets with low nutritional value and little variety have been increasingly consumed, contributing to the growing problems of nutritional deficiencies and chronic diseases as a cause of malnutrition and unbalanced diet (6). Given this panorama, there is a need for advances in knowledge about the composition and beneficial properties to the health of native species that are neglected and underutilized (7). The use of these species can increase the number of native foods currently used, considering the reduction of problems related to the food and nutritional security, as well as the strengthening of conservation and sustainable management of biodiversity (5, 6).

South America presents a rich biodiversity of fruits, however, native fruits culture in this area has not been so well developed; also, its properties were not thoroughly studied too. Several features have been reported in the literature due to its secondary metabolites and their potential effects on human health. Numerous epidemiological studies indicated that a rich diet in fruits and vegetables has been associated with a lower risk of several chronic conditions, including obesity, cancer, and cardiovascular diseases (8, 9).

Fruits present both micro and macronutrients that contain vitamins, minerals, and fibers; however, its biological beneficial properties have been attributed mainly to the presence of bioactive compounds (10). Data suggests that the consumption of these fruits has been associated with a lower incidence of chronic diseases due to the chemopreventive and antioxidant properties of bioactive compounds, such as vitamin C, polyphenols, minerals, etc. (11, 12). These constituents are profoundly valuable due to their antioxidant and anti-inflammatory features. The antioxidant activity of these compounds is manifested by their scavenging ability against reactive oxygen species (ROS) (13).

In Paraguay, several fruits from native plants have been considered, from ancient times, to have a wide range of health benefits. Among them, Yvapurú is one of the most recognized one. The use of native South American fruits has been claimed internationally since the elevated presence of antioxidant compounds has been proved with a consequently high beneficial effect on human health (14, 15). The healthy properties of these fruits may rely on their polyphenolic compositions, since these compounds are found in significant proportions in such fruits. Among these, anthocyanins are known to be the most common type and have been reported to possess antioxidant and anti-inflammatory properties and happen to have the highest concentration among berries (16, 17).

Besides, Yvapurú is popularly used to treat many diseases such as asthma, throat inflammation, and gastrointestinal and cardiovascular disturbances (18, 19). Yvapurú´s peels present antioxidant, anti-inflammatory, and analgesic effects, reduced blood cholesterol and obesity-associated insulin resistance, improved glucose levels and lipid markers, and reduced adipose tissue inflammation, weight gain, dyslipidemia, and hepatic steatosis in animal models (20-24). Therefore, this study aimed to evaluate the nutritional composition, phytochemical screening, total polyphenol content, antioxidant capacity and the identification of bioactive compounds.

Materials and Methods

Fruits of the Yvapurú tree were collected between the months of October and December 2019 after botanical identification, which in this case corresponds to ¨*Plinia cauliflora*¨. For this, a plant of good size, leafy, old and with a good sanitary aspect, located in the San Antonio neighborhood, of the city of Ita, of the Central Department of Paraguay, was selected. The fruiting season was expected, which normally occurs between the months of September to November, with some variations depending on the microclimate of the place and other conditions of the plant. Approximately, two kilograms of fruits were collected. Half kilogram was stored at 4ºC for the analyses that were carried out immediately. The rest were lyophilized as whole fruits, peel and pulp (half kilogram each).

To assess the nutritional chemical composition, the following macronutrients were evaluated; carbohydrates by Anthrona method; proteins by Kjeldahl method; and lipids by Soxhlet method. The results were expressed in grams of macronutrient per 100 grams of sample analysed as described before (25). For phytochemical screening, 10 g of pulverized sample were weighed. Then, 20 mL water-methanol mixture (1:10) with 20 mL of petroleum

ether were added to the sample in a covered flask for light isolation. Subsequently, it was placed on a shaker at 150 rpm for 1 h. After that time, the supernatant was placed in a separation funnel to obtain two phases including methanol-water and oily (ethereal), for the separation of (i) phenols, (ii) flavonoids, (iii) saponins, (iv) alkaloids, (v) steroids. and triterpenoids (vi) leucoanthocyanidins and (vii) quinones (26).

To determine phenols from the methanol-water extract, two qualitative identification tests were carried out including one colorimetric and the other by thin layer chromatography. For the rapid colorimetric test, the ferric chloride (FeCl3) method was used for its identification. A color change to dark blue indicates the presence of pygallic (water-soluble) phenols or tannins. If the change is dark green, it indicates the presence of catechol-type phenols or tannins (flavonoids or condensed tannins). The identification of phenols by thin layer chromatography (TLC) was undetaken using TLC plates (7×4 cm) (Macherey-Nagel, DC-Fertigfolien Alugram, 907189). The mobile phase used was petroleum ether:ethyl acetate:formic acid (40:60:1). Once the plates were run, they were taken under UV light to the length's wavelengths of 254 and 365 nm to observe the number of fluorescent compounds. Subsequently, the revealing agent FeCl3 at 10% was used. It was left to rest for 5 min and placed on an electric grill at 110ºC for a period of 10 min. Finally, the Retention Factor (Rf=solvent distance/sample distance) of each compound found was obtained.

To determine flavonoids, the extract used was methanol-water and was conducted by TLC. The plates had the same characteristics and the same procedure as for phenols were utilized. The mobile phase was ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27). Under UV light at a wavelength of 254nm, blue-, green-, and yellow-colored spots are observed and at 365 nm, dark blue colored spots are observed. Quercetin was used as a positive control.

To identify saponins, the foam test was applied. The extract was diluted 9:1 by taking 1 mL of the methanol-water extract, plus 9 mL of distilled H_2O, placing it in an assay tube glass with lid (13×100 mm). It was shaken vigorously for 30 s, preferably by hand. It was left to stand for 15 min. If the foam height is <5 mm: (-), it is considered negative, it does not contain saponins; about 5–10 mm: (+) argues for moderate content; a height >15 mm: (+++), is attributed to a high content of saponins.

To test for alkaloids, Dragendorff test was used. Three milliLitters of the methanol-water solution were taken, followed by adding 4 drops of ammonia. After bringing it to dryness state, 3 drops of acetic acid and one drop of distilled water were added, and concentrating the solution on an electric iron was undertaken. Drops of this solution were placed on filter paper and covered with drops of Dragendorff's reagent. A change from orange to red or pink suggests the presence of alkaloids.

To identify steroids and triterpenes, the Lieberman-Buchard test was utilized. For this test, the oily extract (ethereal) was used. Totally, 1 mL was taken and placed in a crucible. After its volatilization in a hood, 4-5 drops of chloroform were added to the crucible, mixed well and distributed by dripping to 4 glass tubes with lids. Subsequently, 2 drops of acetic anhydride were added to each tube. With all the tubes covered to prevent evaporation of the solvents and under a hood, a drop of sulfuric acid was added with a Pasteur pipette to only 3 of the tubes so that one of them served as a negative control. A blue or green coloration=steroids; red, pink or violet=triterpenes; and pale yellow=saturated steroids or triterpenes.

For leucoanthocyanidins**,** Rosenheim reaction was applied, while 2 ml of Fraction C were dried and taken up with the same volume of 1% HCl in water. A total of 1 ml of concentrated HCl was added, mixed and heated in a water bath for 10 minutes. Then cooled, a small volume of amyl alcohol was added, and shakes gently. The colour of the amyl phase was finally Observed. The appearance of coloration from crimson to pale pink indicates the presence of leukoanthocyanidins.

For quinones, the direct Borntrager reaction was performed. Totally, 3 ml of Fraction B was shaken gently with 5 ml of 5% NaOH and the coloration was investigated. The presence of a reddish or yellow aqueous phase with red fluorescence indicates the presence of quinones. Regarding the total polyphenol content, five Yvapurú's fruits were taken, later they were cut into halves for seed removing and later, the pulp and rind were stepped on in a glass mortar until obtaining a homogeneous paste (the homogeneous paste was the pulp and peel representative part) for the polyphenol's determination as previously explained (26).

Totally 100 mL of a 50% solution of ethanol of the crude extract of Yvvapurú pulp and peel (1g of sample) was prepared, and the preparation was placed to the sonicator for 30 minutes. Subsequently, the extract was filtered in a 100 mL flask. An aliquot was taken from each of the prepared solutions in different 10 mL volumetric flasks. Then 2000 µL of water and 200 µL of the Folin-Ciocalteu 2N reagent (analytical grade, Merck) were added. It was stirred and later allowed to stand for 5 minutes. In next step, 1500 µL of a 20% Na_2CO_3 aqueous solution

was added to reach a 10 mL final volume with water as solvent. They were shaken and allowed to stand for 1 hour in a dark place. After the time elapsed, the absorbance was read at 760 nm on a UV visible spectrophotometer.

A total of 1000 µL of methanol was used as blank in order to suppress the own absorbance. For quantification, a standard comparison was made with solutions of gallic acid, preparing first a gallic acid stock solution with methanol (100 µg.mL-[1]). From this solution, dilutions were prepared taking 10, 50, 75, 100, 150 and 200 µL in different flasks and proceeded in the same way as in the samples, with a final volume of 10 mL. From the results, a calibration curve was calculated to determine the equivalent concentration of gallic acid in the sample tubes. The results were expressed as mg gallic acid equivalents (GAE, g-[1]); values were presented as the mean of the analyses performed in triplicate ± standard deviation (SD).

The antioxidant capacity in whole fruits was determined through the H-oxygen radical absorbance capacity (ORAC) method. The H-ORAC assay was performed according to Ou *et al.* (27), and Gancel *et al.* (28). Briefly, the 2,2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH) is a source of peroxyl radicals and induced oxidation assay by measuring fluorescein signal in a spectrofluorometer (Synergy™ HT Multi-Mode Microplate Reader model; Biotek Instruments Inc, Winooski, USA), with a 96-well polypropylene plate. Fluorescence is measured at 565 nm with the excitation wavelength at 540 nm. AAPH (1.34 mM) is used as peroxyl radical generator and fluorescein (61 nM) was used as target; fluorescence decay is an indicator of produced damage by the peroxyl radical. The ORAC results were calculated based on the calibration curves obtained in each run. Results were expressed in micromoles of trolox equivalents (TE) per 100 grams as the average of three replicates for each extract. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis-2-methyl-propion-amidine-dihydrochloride (AAPH), and fluorescein were obtained from Sigma Aldrich (St. Louis, MO, USA).

To identify bioactive compounds, once the samples were received, they were immediately frozen at -80°C and subsequently lyophilized to constant weight. The lyophilized samples were individually ground in a mortar to a fine powder and kept at –20°C until use. For extraction of bioactive components, oscillatory shaking at 900 oscillations/min (Vibromatic, Selecta, Spain) for 60 min was carried out. To 1 g of the dehydrated and pulverized sample, 10 mL of extractant composed of methanol (77%), water (20%) and formic acid (3%) with 5 ppm of hesperidin were added, which was used as an internal standard. The solid–liquid system was centrifuged for 5 min at 1480 ×g to separate the solids from the extract. The operation was undertaken three consecutive times to ensure an extraction yield greater than 95%. The extracts obtained from the three extractions were mixed and filtered before analysis by liquid chromatography-mass spectrometry. This extraction was carried out in triplicate for all samples.

To quantify bioactive components in extracts by Ultra Performance Liquid Chromatography coupled with hybrid triple quadrupole linear ion trap tandem mass spectrometry (UPLC-ESI-QqQLIT-MS/MS) method, the characterization of the extracts was performed by liquid chromatography (Agilent Technologies, 1200 series) coupled to mass spectrometry (LC-QqQ MS/MS) using a triple quadrupole detector (Agilent Technologies, model 6460). Chromatographic separation was performed using a C18 reversed phase column (Zorbax Eclipse Plus C18 Rapid HD Resolution 3.0×150 mm, 1.8 µm), using water (phase A) and acetonitrile (phase B) as mobile phases, both with 0.1% of formic acid as an ionizing agent. The elution gradient was as follows: 0 to 1 min, 4% phase B; 1 to 6 min, increases from 4% to 40% phase B; from 6 to 10 min, it increases from 40% to 100% of phase B; for 10 to 20 min, keep in 100% phase B to ensure elution of all components of the sample. Subsequently, the column was equilibrated to the initial conditions for 15 min, before the next analysis. The flow of the mobile phases was 0.25 mL/min and the injection volume used was 2 µL. Mass spectrometric analysis was performed using an electrospray ionization source.

Results

The results of the nutritional chemical composition are expressed in g/100 grams of sample in Table 1. The phytochemical analysis revealed that all the fruits presented phenols and triperthenoids. Regarding the total content of polyphenols, the analysis showed that Yvapurú contained an amount of 24.4 mg/g. The determination of the antioxidant capacity, showed that Yvapurú fruit presented 10.200±334 µmol/100 g. The quantification of bioactive compounds demonstrated that the Yvapurú shell samples were the ones with the

Table 1: Macronutrient contents in Yvapurú.

Macronutrient (g/100 g of sample	Yvapurú fruit
Carbohydrates	47.8±2.3
Proteins	7.24±0.15
Fat	1.28±0.34

Table 2: Identification of bioactive compounds (mg/kg).

Fruit		Cyanidin 3-O-glucoside	Delphinidin 3-glucoside	Oenothein B	Quercetin 3-O-rhamnoside	Myricetin 3-O-rhamnoside
Yvapurú	Shell	1759.2±73.4	79±3.5	141.6±2.4	66.4±0.5	21.4±0.4
	Pulp	40.4±6.8	N/D	169.3±11.5	N/D	N/D

*N/D: Not detectable.

highest levels of cyanidin 3-O-glucoside, between 1443 and 1759 mg/kg. In the case of pulp samples, cyanidin 3-O-glucoside was found from Yvapuru in a range of 7 to 40 mg/kg.

On the other hand, only delphinidin 3-O-glucoside was found in the shell samples from Yvapuru (60 mg/kg). The Quercetin 3-O-rhamnoside was identified in the shell Yvapurú as 64.4 mg/kg. The myricetin 3-O-rhamnoside was identified in the shell in the Yvapurú as 21,4 mg/kg. Finally, oenothein B was detected in the samples of the Yvapuru shell as approximately 58 mg/kg. The concentration was the average expressed in mg of the bioactive compound per kg of sample. The results of the quantification of the different samples were illustrated in Table 2. The identified analytes can be seen in Figure 1 and 2.

Discussion

About nutritional chemical composition, since there are no nutritional chemical composition studies for Yvapurú fruit species, the macronutrient values were compared with those of the ¨blackberries¨

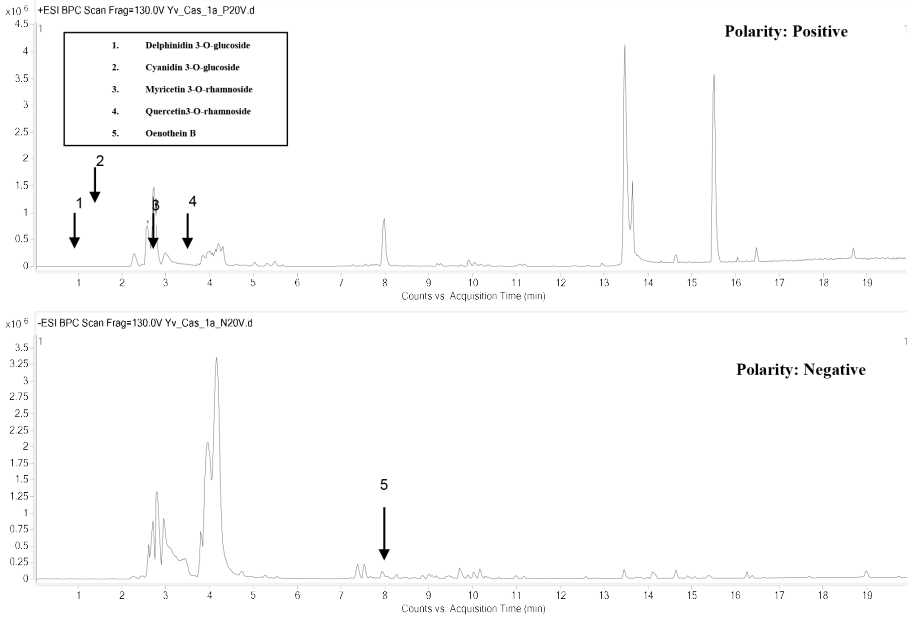

Figure 1: Analytes in the shell of Yvapurú.

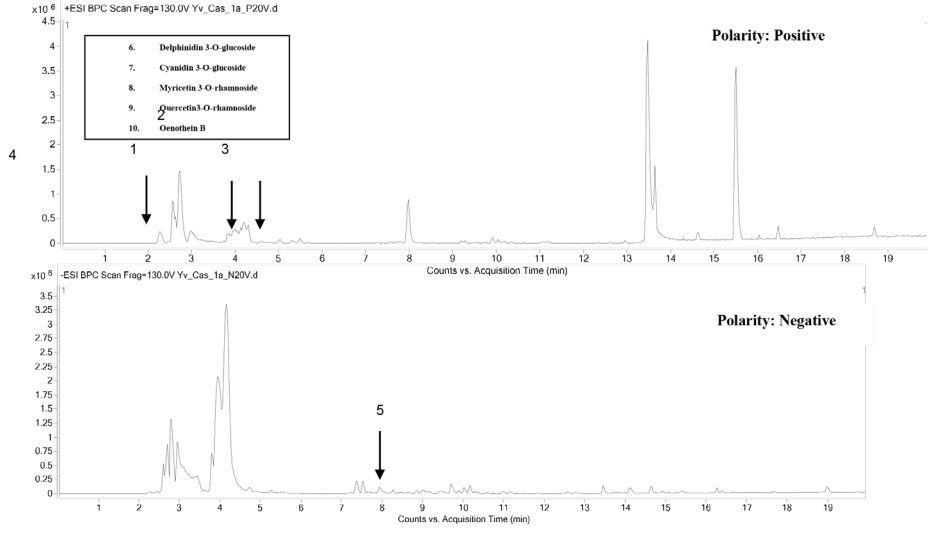

Figure 2: Analytes in the pulp of Yvapurú.

(*Rubus ulmifolius*) fruits, because they are considered standard among dark purple fruits. Regarding the amount of carbohydrates, da Silva *et al.*, found 26.2 g/100 grams of sample in blackberry fruits, almost half of what was found in Yvapurú fruits (29). The amount of protein in the da Silva *et al.*'s Study was 2.4 g/100 grams of sample, a lower figure than found in Yvapurú´s fruit (7.24 g/100 g). Regarding the fat content, da Silva's *al.*'s Study observed an amount of 1.22 g/100 g, similar to the amount of fat in Yvapurú (1.28 g/100 g) (29).

A systematic review on *P. cauliflora* phytochemical components of Brazilian origin, revealed the presence of phenolic compounds, flavonoids and terpenoids in whole fresh fruit, compounds that were present in the fruits of this study. Phenolic compounds and flavonoids are known for cardio protective effects (30). Also, leucoanthocyanidins were present in the Yvapurú, a result that agrees with that found in the fruit peel of the Brazilian species of *P. cauliflora* (31).

Regarding the total phenolic compounds, Souza-Moreira *et al.* found a total content of >45 in the Brazilian Yvapurú species, more than the Paraguayan Yvapurú specie (24.4 mg/g.) (32). This value of total polyphenols gives us the indication that this fruit is rich in antioxidants. One of the functions of these compounds would be a chronic non-communicable disease preventive by its consumption. The quantification of total polyphenols is a direct way to estimate antioxidant content in a food matrix.

Taking into account the study by Yoh *et al.*, who found values of antioxidant capacity between 9.776 and 37.845 μmol/100 g in blackberries, so the values of this research were relatively high (10.200±334) µmol/100 g. (33). Cyanidin reduces lipid accumulation in adipose tissue, improves insulin sensitivity, improves adiponectin mRNA levels, suppresses production of nitric oxide and inflammatory cytokines in colorectal adenocarcinomas, inhibits oxidative stress and neuroinflammation, improves cell degeneration and activates neurotrophic factor signalling (34, 35). In this study, we found that the Yvapurú shell samples were the ones with the highest levels of cyanidin 3-O-glucoside, between 1443 and 1759 mg/kg.

Delphinidin inhibits lipid accumulation, suppresses proliferation induced by oxLDL, inhibits endothelial cell apoptosis, decreases the production of reactive oxygen species (ROS), significantly inhibits platelet activation and attenuates thrombus growth, which probably contributes to its protective functions against thrombosis and CVD (36). We found this compound in the shell samples from Yvapuru (60 mg/kg). Quercetin was shown to inhibit osteoclastogenesis, osteoblast apoptosis, oxidative stress, and the inflammatory response while promoting osteogenesis, angiogenesis, antioxidant expression, adipocyte apoptosis, and osteoclast apoptosis (37). This research identified this compound in the shell Yvapurú in 64.4 mg/kg.

Myricitrin has a potential capacity to accelerate the fibroblastic and remodeling phases in the wound repairing process, it promotes fibroblast migration, demonstrating a twice higher rate of wound closure and it is a powerful scavenger of free radicals, which in excess are harmful to health (38, 39). We found in the shell in the Yvapurú (21,4 mg/kg) of this compound.

Enotein B inhibits the proliferation of lung cancer cells, has a high antioxidant capacity and to eliminate free radicals, protects macrophages from oxidative damage, increases the production of antioxidants, and reduces neuroinflammation in the brain during systemic inflammation (40). The amount that we found in this research was approximately 58 mg/kg, in the Yvapuru shell.

Conclusion

We can conclude that the antioxidant properties and its content of bioactive compounds make the Yvapurú fruit a healthy option in terms of its consumption; in addition, being a leafy tree, its cultivation would be the beautification of places. In addition, the massive cultivation of this fruit tree could provide the raw material to consume its fruits in different ways. The next step in this line of research would be to conduct clinical trials in people with metabolic disorders to see the impact on their health. The results obtained in this work open a wide range of opportunities in terms of food safety, economic possibilities for the agri-food sector and the doors to enter the so-called "nutraceuticals", which are a line of research now open to take advantage of the fruits of species typical of our land, all this, based on their properties and benefits that their consumption supposes for human health.

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Conflict of Interst

None declared.

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