INTRODUCTION

Worldwide, nopal has become a valuable crop due to its health benefits, ease of cultivation, marketing, and climate adaptation (Aruwa et al., 2018). Nopal (Opuntia ficus-indica (L.) Mill) belongs to the Cactaceae family that comprises about 1,500 species (El-Mostafa et al., 2014), some of these species are Opuntia: basilaris, chlorotica, engelmannii, fragilis, humifusa, leucotricha, macrocentra, macrohiza, dillenii, santa-rita, stricta (Majdoub et al., 2001). Its cultivation represents a major source of income for farmers living in semi-arid regions (Bayar et al., 2016). Nopal can grow in South America and other dry areas such as Africa, Australia, Southern Europe, and Asia (Khouloud et al., 2018; Majdoub et al., 2001). Nevertheless, Mexico accounts for 90% of the world’s production and represent the largest supplier to the United States, Canada, Japan, and European countries. Per capita consumption of nopal in Mexico is 6.4 kg (FAOSTAT, 2016).

Nopal is one of the most consumed species due to its nutritional value (Majdoub et al., 2001); furthermore, recent trends in healthy food consumption aroused scientists’ interest to study the effects of...
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nopal polyphenolic compounds in oxidative stress-related diseases (Scalbert et al., 2005). This work describes the nopal as a potential source of polyphenols and the main factors affecting their analytical identification. In addition, we highlight the importance of the relationship structure function in promoting health through cladodes consumption.

2 Nopal: Morphological Description

The hierarchical structural organization evaluates the structure functioning at different dimensional scales (macrostructure, microstructure, and nanostructure) (Gibson, 2012). Figure 1 shows a model of the hierarchical structural organization of the nopal, which contributes to its morphological description.

At the first level (macrostructure), nopal has three components which are as follows: flowers, prickly pears fruits, and leaves (botanically called cladodes) (Salehi et al., 2019). The flowers are pear-shaped, which allows insect pollination (Small & Catling, 2004). The prickly pears are usually ovoid and spherical, often green, yellow, or bright red. They have a high number of seeds, and a protective shell covered with small spines. This gives them an important role in the genetic diversity and distribution of the species (Carrillo et al., 2017).

The leaves or cladodes (Figure 1a) are ovoid or elongated racquet-shaped, with 30–60 cm in length depending on the water and nutrients available (Criminna et al., 2019). In Africa, cladodes are exclusively used for animal feeding (De Albuquerque et al., 2019; Mounir et al., 2020); while in Japan, they are hydroponically cultivated for human consumption (Horibe, 2018), as a medicinal plant for diabetes and hypercholesterolemia (Santos-Zea et al., 2011). Cladodes have areolas from where flowers, fruits, and thorns grow. One to five large, hard spines, and multiple smaller ones (glochidia) protect cladodes against light reflection, water loss, and herbivores predation (Marin-Bustamante et al., 2018).

The epidermis (Figure 1b) contains numerous stomata (Figure 1c) that control photosynthesis and respiration (Salem-Fnayou et al., 2014). An inner tissue called chlorenchyma (CH) constitutes the second hierarchical level (microstructure), which consists of green plastids and abundant starch. The vascular tissue (V) located at the chlorenchyma tissue and the nucleus tissue (N) junction serves as a water and nutrient transporter into the plant, allowing the tissue to function as water storage for long periods of drought (Ginestra et al., 2009). The colorless central core tissue contains reserves of carbohydrates, proteins, and polyphenols (Feugang et al., 2006).

At the third hierarchical level (nanostructure), the macro cellulose fibers provide structure to the cell wall (Figure 1e). Alongside the tissues, calcium oxalate crystals are found (decreasing in content as cladodes mature) making calcium more bioavailable in younger cladodes (Contreras-Padilla et al., 2016), which are consumed as vegetables in different stages of maturation ranging from 30 to 90 days (Hernandez-Becerra et al., 2020; Marin-Bustamante et al., 2018). Finally, on the fourth hierarchical level, we find the cellulose molecular structure (Figure 1f) (Ventura-Aguilar et al., 2017).

3 Cladode: Composition and Biological Activity

Cladode chemical composition may vary according to soil factors, cultivation season, and plant age (Table 1). The primary metabolites of cladodes are water, carbohydrates, and proteins. The carbohydrates in cladodes are divided into two types: (a) structural ones that are part of the cell wall, as cellulose (21.6 wt%), hemicelluloses 8.19%, and lignin (3.6 wt%) (López-Palacios et al., 2016; Scaffaro et al., 2019), and (b) the storage carbohydrates constituted by monosaccharides such as arabinose, galacturonic acid, glucuronic acid, galactose, glucose, xylose, rhamnose, mannose, and fructose (Rodríguez-González et al., 2014). Polysaccharides from Opuntia

![Hierarchical structural organization of nopal](image-url)
Ficus-indica (L.) Mill plants build molecular networks with the capacity to retain water, thus they act as mucoprotective agents (Di Lorenzo et al., 2017). Mucilage is the main polysaccharide of cladodes, it contains polymers of β-β-galacturonic acid bound in positions (1–4) and traces of R-linked L-rhamnose (1–2) (Figure 2) (Quinzio et al., 2018). Mucilage regulates both the cell water content during prolonged drought and the calcium flux in the plant cells (Hernández-Urbiola et al., 2010). In the food industry, mucilage is used as an additive, an emulsifier, and an edible coating to extend the shelf life of food products (Medina-Torres et al., 2013).

Cladodes contain around 6.7%–11.73% of protein (Table 1). Amino acids such as alanine, isoleucine, and asparagine are found in young cladodes, whereas threonine prevails only in mature cladodes (Figueroa-Pérez et al., 2018). Young cladodes have a higher protein content than mature cladodes, which may be related to the increased metabolic activity in the early stages of maturation (Nuñez-López et al., 2013). Furthermore, analyses of plant extracts of the Cactaceae family identified several enzymes (e.g., lipases, proteinases, and glucosidases) (Guevara-Figueroa et al., 2010), and a large content of minerals (23.05%).

Over the years, Mexican people have developed several chronic degenerative diseases such as obesity, diabetes, and cardiovascular diseases (Aparicio-Saguilán et al., 2015). Traditional Mexican medicine recommends consuming cladodes due to their bioactive compounds’ effects on health (Table 2); for example, the ability of polyphenols to eliminate free radicals (De Santiago et al. 2019; Filannino et al., 2016; Kim et al., 2016; Petruk et al. 2017).

Avila-Nava et al. (2014) assessed the antioxidant capacity of cladodes both in vitro and in vivo, by evaluating the consumption of cladodes for 3 days (300 g/day) in healthy subjects aged 20–30 years.

**TABLE 1** Chemical composition of nopal cladodes

| Carbohydrates | Proteins | Lipids | Crude fiber | Ash | Reference                  |
|---------------|----------|--------|-------------|-----|---------------------------|
| 42.94         | 7.07     | 2.16   | 7.07        | 17.65 | Hernandez-Urbiola et al. (2010) |
| 61.4          | 6.7      | 0.1    | 15.0        | 17.3  | Guevara-Figueroa et al. (2010) |
| 38.0          | 11.2     | 0.69   | 5.97        | 14.4  | Astello-Garcia et al. (2015) |

Note: —, No determinate; DW, dry weight.

**FIGURE 2** Structural proposal of the Opuntia ficus-indica mucilage

Phenolic acids
- Myricetin-glu, quercetin-glucoside, kaempferol-glucoside, isorhamnetin-glucoside, quercetin-rhamnose-hexoside-rhamnose, kaempferol-di-rhamnose-hexoside, isorhamnetin-rhamnose-rutinoside, quercetin-hexoside-pentoside, isorhamnetin derivative, quercetin-3-O-rutinoside, quercetin-hexoside, kaempferol-rutinoside, isorhamnetin-rutinoside, isorhamnetin-C-hexoside, isorhamnetin pentoside, trihydroxy-methoxy-flavonol, naringin, rutin, nicotiflorin

Flavonols
- Naringenin-hexoside, naringin gaulacil (8-O-4) syringyl (8-8) gaulacil-hexoside, 3,3’,4’,5’,7pentahydroxy-flavanone

Flavanones
- Luteolin-glucoside, apigenin-glucoside, isoflavonoid

Flavones
- Cyanidin-glucoside, pelargonidin-glucoside, petunidin-glucoside, delphinidin-glucoside, malvidin-glucoside
with a body mass index (BMI) <25 kg/m². The results showed an increase in the antioxidant activity of blood (↑5%) and plasma (↑20%). The polyphenols quercetin, isorhamnetin, and kaempferol were identified by high-performance liquid chromatography (HPLC). The authors concluded that consuming cladodes can reduce pathologies associated with reactive oxygen species.

Additionally, Petruk et al. 2017 found that eucomic and piscidic acids obtained from cladodes polyphenols were responsible for antioxidant activity and produced a protective effect against apoptosis of human keratinocytes induced by UVA. Scholars classified cladodes as a functional food and a prebiotic since they modify the gut microbiota, reduce metabolic endotoxemia, and other obesity and metabolic syndrome biochemical abnormalities (Angulo-Bejarano et al., 2014; Mercado-Mercado et al., 2019; Sanchez-Tapia et al., 2017). Cladodes have antimicrobial, anticancer, and antiidiabetes activity and protective effects on hypertension, hypercholesterolemia, rheumatic pain, antulcerogenic activity, gastric mucosa diseases, and asthma (Tahir et al. 2019). These beneficial health outcomes are attributed to some components of cladodes: polyphenols (phenolic acids, flavonoids, and anthocyanins), β-carotene, oligosaccharides, polysaccharides, sterols, lignans, saponins, and some vitamins such as E and C (du Toit et al. 2018).

4 | POLYPHENOLS: EXTRACTION AND IDENTIFICATION METHODS

Polyphenols constitute one of the largest groups of secondary plant metabolites (Galanakis et al., 2018; Mirali et al., 2017). They contain one or more hydroxy groups linked to a benzene ring and have an essential function in the defense against plant pathogens and abiotic stressors (López-Romero et al., 2014). Figure 3 shows the main polyphenols identified over time in cladodes by different analytical techniques (Antunes-Ricardo et al., 2014, 2015, 2017; Astello-Garcia et al., 2015; De Santiago et al., 2018; Msaddak et al., 2017; Rocchetti et al., 2018). Young cladodes have a higher content of polyphenols than mature ones (Figueroa-Pérez et al., 2018).
4.1 | Extraction techniques for the analysis and characterization of polyphenols

Due to the high fiber content of cladodes (Table 1), other minor compounds (of equal biological importance), such as polyphenols, have not been studied deeply. Therefore, we reviewed the methods to extract and characterize polyphenols. Types of extraction methods include liquid-solid extraction (a procedure that consists of grinding, defatting, solvent extraction, centrifugation, filtration, evaporation, and drying) (Yang et al., 2018) methanol/water/acid, methanol/acetone/water, and methanol/formic acid-based techniques and are optimized by varying methanol concentrations between 50% and 80% (Table 3). For instance, Antunes-Ricardo et al. (2014, 2015, 2017) extracted polyphenols with 4 N NaOH (1:10, m/v) at 40°C. Another study carried out by column chromatography, showed that a combination of 45°C and airflow allowed optimal preservation of phenols and flavonoids (Medina-Torres et al., 2011). The cladodes extracted by ethanol exhibited good solubility in polar solvents because the polar compounds act as scavengers against reactive oxygen species (Bonilla Rivera et al., 2017). Lastly, the Soxhlet and maceration method conducted by Ammar et al. (2015) in which variability in the extracts yields was attributed to the different polarities of the solvents used; in particular, the methanol and water extract produced the highest extraction yields.

Obtaining polyphenol-rich extracts requires sample purification by column chromatography (Nemitz et al., 2015). Chromatography is a physical separation method based on differential migration of the sample components carried by the mobile phase through a stationary phase arranged in a column (Granato et al., 2016). Four types of chromatography can be applied to determine the polyphenolic profile of crude extracts from biological materials: high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and capillary electrophoresis (CE) (Agatonovic-Kustrin et al., 2020; Gadioli et al., 2018).

The highest extraction yields were obtained when using C18 reversed-phase HPLC columns (with inner diameter 2–250 mm; particle size 1.8–2.5 μm) and a mobile phase composed of methanol or acetonitrile under isocratic elution or gradient elution (i.e., water and 0.1%-10% acetic or formic acid) conditions (Table 3). However, a factor to consider is the production of raw extracts, in which the management of parameters such as extraction time, temperature, and solvent composition influence the concentration and types of compounds obtained.

4.2 | Identification of polyphenols

Several authors have identified polyphenols in cladodes through HPLC and UHPLC because they maximize polyphenol identification accuracy (Tan & Fanaras, 2018). Hence, these techniques lead the separation methods for polyphenols analysis (Table 3).

A study conducted by Petruk et al. (2017) with extracts from Opuntia ficus-indica var. saboten found three phenolic acid derivatives: piscidic, eucomic, and 2-hydroxy-4-(4-hydroxyphenyl)-butanoic acid. Astello-García et al. (2015) identified polyphenols via LC-MS according to retention time, UV spectra, and mass (m/z). Through the aglycone fragment, they examined the structure of each flavonoid by characterizing quercetin ([M]+ m/z 301), isorhamnetin ([M]+ m/z 315), kaempferol ([M]+ m/z 285), and luteolin ([M]+ m/z 285).
### Analytical methods for the determination of polyphenols in cladodes

| Opuntia species | Extraction method | Analysis | Compounds identified | Reference |
|-----------------|-------------------|----------|----------------------|-----------|
| *Opuntia ficus-indica* (L. Mill.) | Alkaline hydrolysis: 4 N NaOH (1:10, m/v) at 40°C for 30 min | Detection method: LC/MS-TOF. Stationary phase: Zorbax SB-C18, 3.0 × 100 mm, 3.5 µm. Mobile phase: A: water–formic acid, B: methanol. Mode: [M]+: 100–1500 m/z | Quercetin glucosyl-rhamnosyl-pentoside, isorhamnetin dihexosyl-rhamnoside, kaempferol rhamnosyl-rhamnosyl-glucoside, isorhamnetin-glucosyl-rhamnosyl-rhamnoside, isorhamnetin-hexosyl-methylpentosyl-pentoside, isorhamnetin glucosyl-pentoside, kaempferol-glucosyl-rhamnoside, isorhamnetin glucosyl-rhamnoside | Antunes-Ricardo et al. (2014) |
| *Opuntia ficus-indica* (L. Mill.) | Solvent extraction: 0.1 g of sample in 2 ml of methanol:acetone:water (5:4:1), 2.5 hr at 4°C | Detection method: LC–MS/MS. Stationary phase: Hydro-RP18, (150 mm × 4.6 mm × 3 mm). Mobile phase: A: acetonitrile/methanol-formic acid, B: formic acid. Chromatograms recorded: λ = 200–600 nm | Eucomic acid, chlorogenic acid, chlorogenic acid derivative, quercetin 3-O-rhamnosyl-(1→2)[rhamnosyl-(1→6)]-glucoside, quercetin 3-O-xilosyl-rhamnosyl-glucoside, quercetin 3-O-dirhamnoside, kaempferol 3-O-(rhamnosyl-galactoside)-7-O-rhamnoside, kaempferol 3-O-(rhamnosyl-glucoside)-7-O-rhamnoside, kaempferol 3-O-robinobioside-7-O-arabinofuranoside, isorhamnetin 3-O-rhamnoside-7-O-(rhamnosyl-hexoside), quercetin 3-O-rutinoside, quercetin 3-O-glucoside,isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside, quercetin 3-O-arabinofuranoside, kaempferol 3-O-glucoside, kaempferol 7-O-neohesperidoside, isorhamnetin 3-O-galactoside | Astello-García et al. (2015) |
| *Opuntia ficus-indica* (L. Mill.) | Alkaline hydrolysis: 4 N NaOH (1:10, m/v) at 40°C for 30 min | Detection method: HPLC-PDA. Stationary phase: Zorbax SB-C18 (9.4 × 250 mm, 5 µm). Mobile phase: A: water–formic acid, B: formic acid. | Isorhamnetin-glucosyl-pentoside, isorhamnetin-glucosyl-rhamnoside, isorhamnetin-glucosyl-rhamnosyl-rhamnoside, isorhamnetin-glucosyl-rhamnosyl-pentoside | Antunes-Ricardo et al. (2015) |
| *Opuntia ficus-indica* f. inermis | Maceration: 25 g of sample, ethanol 100%, 24 hr | Detection method: LC-HRESIMS. Stationary phase: RP Pursuit XRs ULTRA 2.8, C18, 100 mm × 2 mm. Mobile phase: A: formic acid-water, B: formic acid-methanol. Mode: [M]+ 100–2,000 m/z | Quercetin, quercetin 3-O-glicoside, kaempferol, kaempferol 3-O-glucoside, kaempferol 3-O-rutinoside, isorhamnetin, isorhamnetin 3-O-glucoside, isorhamnetin 3-O-neohesperidoside, 3,3′,4′,5,7-pentahydroxy-flavanone, p-coumaric acid, zataroside-A, indicaxanthin, 8-sitosterol | Msaddak et al. (2017) |
| *Opuntia ficus-indica* (L. Mill.) | Solvent extraction: 4 g of sample, methanol 50%, 2 hr | Detection method: HPLC-DAD. Stationary phase: Kinetex C18, 5 µm RP 250 × 4.60 mm. Mobile phase: A: water–formic acid, B: acetonitrile. Chromatograms recorded: Phenolic acids: λ = 256–325 nm, flavonoids: λ = 360 nm | Quercetin, kaempferol, isorhamnetin, ferulic acid, 4-hydroxybenzoic acid | De Santiago et al. (2018) |
| *Opuntia ficus-indica* | Agitation: 4 g of sample, 0.1% formic acid in 80:20 (v/v) methanol/water, 25,000 rpm for 3 min | Detection method: UHPLC-ESI-QTOF-MS; Stationary phase: Agilent Zorbax eclipse plus C18, 50 × 2.1 mm, 1.8 µm. Mobile phase: A: water, B: methanol-formic acid-ammonium formate. Mode:[M]+ 50–1000 m/z | Luteolin-glu, apigenin-glu, isoflavonoid, myricetin-glu, quercetin-glu, kaempferol-Glu,isorhamnetin-Glu, furfurans, dibenzylbutyrolactone, alkylphenols, hydroxybenzaldehydes hydroxycoumarins tyrosols, hydroxybenzoics, hydroxypheylpropanoics, hydroxycinnamics | Rocchetti et al. (2018) |

(Continues)
| Opuntia species | Extraction method | Analysis | Compounds identified |
|----------------|-------------------|----------|----------------------|
| *Opuntia ficus-indica* (L.) Mill. | Solvent extraction: 200 mg of sample, methanol acidified with formic acid, Sonicated for 25 min. | Detection method: UHPLC-ESI-MS n. Stationary phase: XSelect HSS T3, (50 × 2.1 mm × 2.5 µm). Mobile phase: A: acetonitrile—formic acid, B: acidified acetonitrile. Mode: [M]−: non-colored phenolics, [M]+: Betalains. | Protocatechuic acid hexoside, myricetin-hexoside, ferulic acid derivative, ferulic acid hexoside, sinapic acid hexoside, quercetin-3-O-rutinoside, quercetin-5-O-rutinoside, kaempferol di-rhamnose-hexoside, syringaresinol, naringenin, naringin. |
| *Opuntia ficus-indica* (L.) Mill. | Maceration: 500 mg in 25 ml aqueous methanol (80%) overnight at 4°C | Detection method: LC/MS-TOF. Stationary phase: Agilent Extended C18 (1.8 µm, 2.1 × 50 mm). Mobile phase: A: water + 0.1% formic acid, B: acetonitrile. Mode: [M]−: 50–1,700 m/z. | Piscidic acid, eucomic acid, isorhamnetin glucosyl-rhamnosyl-rutinoside, isorhamnetin-2-glucosyl-rhamnosyl-pentoside, rutin, narcissin (isorhamnetin rutinoside). |
| *Opuntia ficus-indica* (L.) Mill. | Solvent extraction: 100% methanol (3 × 2 L) | Detection method: HPLC-PDA-MS/MS. Stationary phase: HS F5 column (15 cm × 4.6 mm, 5 µm). Mobile phase: A: water + 0.1% formic acid, B: acetonitrile. Mode: [M]−: 50–1,700 m/z. | Quinic acid, malic acid, piscidic acid, diferuloyl-syringic acid, p-coumaric acid, 3-O-glucoside, 7-glucosyl-oxy-5-methyl flavone glucoside, quercetin-pentosyl-rutinoside, kaempferol rhamnosyl-rutinoside, isorhamnetin glucosyl-rutinoside, rhamnetin 3-O-glucoside, isorhamnetin coumaroyl-rutinoside, rhamnetin, isorhamnetin, diosmetin, tricin, hydroxyl octadecadienoic acid, eicosanoic acid, isomer of behenic acid. |

Note: UHPLC-PDA: high-pressure liquid chromatography equipped with a photodiode array detector; UHPLC-ESI-QTOF-MS: high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry; HPLC-PDA-MS/MS: High-performance liquid chromatography-photodiode array-electrospray ionization tandem mass spectrometry; LC/MS-TOF: liquid chromatography coupled to a time-of-flight mass spectrometer; UHPLC-ESI-MS n: ultrahigh-performance liquid chromatography with electrospray ionization tandem mass spectrometry; LC-MS/MS: liquid chromatography-tandem mass spectrometry; HPLC-DAD: high-performance liquid chromatographic diode array detector system; LC-ESI-QTOF-MS: liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry.
Similarly, Antunes-Ricardo et al. (2015) found isorhamnetin glycosides by HPLC-PDA. Rocchetti et al. (2018) detected 89 flavonoids—mostly the glycosidic forms of kaempferol, isorhamnetin, and quercetin—and 54 phenolic acids in cladodes. This was the first evaluation that includes the phenolic profile in cladodes using UHPLC-ESI/QTOF-MS. Msadak et al. (2017) studied an ethanolic extract of cladodes utilizing LC-HR-ESI-MS; they found 9 flavonoids and 2 phenolic acids. Furthermore, Mena et al. (2018) identified flavanones and lignans by UHPLC-ESI-MS and observed a higher polyphenol content in young cladodes compared with matures ones, which could be attributed to physiological modifications.

Spectrometry-based techniques are a powerful and fast tool to accurately differentiate compounds in food matrices. However, it is unreliable when quantifying polyphenols due to the lack of availability of reference standards. Among the analytical methods compared in this review, UHPLC-ESI/QTOF-MS exhibited an outstanding performance to identify the main polyphenolic classes and subclasses in cladode extracts. Simultaneously, it detected multiple compounds based on the mass-to-charge ratio (m/z) of a molecular ion ([M – H]+) and the characteristic production for each polyphenol.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

The present study reviewed the structure function of cladodes, which may provide an nutritional and functional value given the properties of their major chemical components. Several studies have shown that polyphenols in cladodes are associated with beneficial effects on human health. Polyphenols can be separated and identified by conducting advanced analytical techniques, which have different advantages associated with the solute-solvent ratio. Here, we described diverse processes in current research to detect polyphenols in cladodes that could be implemented in future technological developments. The forthcoming research should focus on obtaining additional information to standardize the analytical methods designed to categorize and quantify the polyphenols in cladodes; and conducting more experimental studies, such as in vivo models, on polyphenol cladode extracts to determine the characterization of nopal biological activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Madeleine Perucini-Avendaño: Conceptualization (lead); Investigation (lead); Writing-original draft (lead). Mayra Nicolás-García: Conceptualization (supporting); Investigation (supporting). Cristian Jimenez: Conceptualization (equal); Investigation (equal); Supervision (lead); Writing-review & editing (supporting). Maria de Jesús Perea-Flores: Formal analysis (supporting); Supervision (supporting). Mayra Beatriz Gómez-Pañüno: Visualization (supporting). Daniel Arrieta-Baez: Visualization (supporting). Gloria Davila-Ortiz: Project administration (lead); Supervision (supporting); Writing-review & editing (lead).

ETHICS APPROVAL

Studies involving animal or human subjects were not required for this review.

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