

Use of plant extracts to control bacterial foodborne pathogens

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Foodborne illness is a common public health problem, affecting the economic development of countries. In the United States of America (USA) it is estimated that 39% of foodborne illness are caused by bacteria, of which non-typhoid *Salmonella*, *Clostridium perfringens*, and *Campylobacter* are the main pathogens involved. In the year 2014 the European Union (EU) reported that 20% of foodborne outbreaks were caused by *Salmonella*, 16.1% by bacterial toxins, and 8.5% by *Campylobacter*. To ensure the production of safe food, synthetic chemical additives are traditionally used. However, natural compounds with preservative activity, known as green additives, have gained prominence in the field of food science as an alternative to traditional additives. Among these antimicrobials, plant extracts have demonstrated antimicrobial effect *in vitro* and *in situ* against foodborne pathogens. Several reports examine bioactive compounds with antimicrobial properties usually present in plants, such as phenolics, alkaloids, terpenoids, and saponins. In this chapter we will discuss the antibacterial activity of plant extracts, the main subclass of active compounds responsible for the antibacterial effect and the application of these extracts in foods. We have analysed and critically discussed around 70 articles covering the latest improvements in the field.

Keywords: foodborne illness; synthetic preservatives; natural compounds

1. Introduction

Foodborne diseases are a serious global health problem. Each year, foodborne diseases cause about 600 million cases of illness, and 420,000 of these people die, including 125,000 children under 5 years of age [1]. This is not just a problem in the underdeveloped world. In the USA, 864 foodborne outbreaks were reported in 2014, resulting in 13,246 cases of illness, 712 hospitalizations, 21 deaths, and 21 food recalls [2]. In 2015, a total of 4,362 foodborne outbreaks were reported in the EU, resulting in 45,874 cases of illness, 3,892 hospitalizations and 17 deaths [3].

Microbial, chemical or physical agents can cause foodborne illness when ingested. Over recent years, bacterial foodborne agents have been the most thoroughly investigated and monitored causes of intestinal infectious disease. Throughout the last decade, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. have persisted, commanding the most research and surveillance attention from government agencies and the most awareness from the food industry. These bacterial pathogens together constitute the greatest burden of foodborne illness for which the etiology is known. Examples of foodborne pathogens, clinical manifestations and associated foods are shown in Table 1 and will be addressed below.

Synthetic chemical additives are traditionally used to control the multiplication of foodborne pathogens [4]. Although these antimicrobials are approved for use in many countries, the recent trend has been the use of natural preservatives due to the adverse effects of chemical additives [5]. In addition, consumer awareness of the problems associated with synthetic additives has generated a profile of "green" consumers who require the absence of additives in foods [4]. In order to find alternatives to synthetic chemical additives, research studies on natural antimicrobials have appeared, and among these, in this chapter, plant extracts stand out. Compounds from plants are divided into two categories: the products of the primary metabolism, such as carbohydrates, proteins, and fats; and the secondary metabolites, such as phenolics, quinones, alkaloids, and terpenoids. Many of these metabolites are involved in plant defense mechanisms against insect and microorganism attack [6], and some secondary metabolites have shown antimicrobial activity [7].

2. Bacterial foodborne pathogens

2.1 *Escherichia coli*

There are several different categories of diarrheagenic *E. coli*, and the most important or most studied are enterohemorrhagic (EHEC), enterotoxigenic (ETEC), and enteropathogenic (EPEC) *E. coli*. There are three other categories, including enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC) *E. coli* [8]. The enterohemorrhagic *E. coli* (EHEC) is capable of causing watery or bloody diarrhea, the latter termed hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS). *Escherichia coli* serotype O157:H7 is the most well-known EHEC strain and can produce Shiga toxin (Stx), hence, it is known as a type of Shiga toxin-producing *E. coli* (STEC). *Escherichia coli* O157:H7 can be transmitted to humans through contaminated food and water, directly between persons, and through contact with animals or their environment. Cattle are the main reservoir of *E. coli* O157:H7, and ground beef is the most frequently identified vehicle of transmission to humans [9, 10, 11].

2.2 *Salmonella* spp.

The genus *Salmonella* belongs to the family *Enterobacteriaceae*, whose members are Gram-negative, facultative anaerobic, straight rods. Within the genus *Salmonella*, currently three species (*enterica*, *bongori*, and *subterranea*) and six subspecies (*arizonae*, *diarizonae*, *enterica*, *houtenae*, *indica*, and *salamae*) are differentiated [12, 13, 14]. There are more than 2600 serovars belong to *Salmonella enterica* subsp. *enterica* [15].

Bacteria of the genus *Salmonella* usually live in the intestinal tract of various warm- and even coldblooded animals and humans [16]. *Salmonella* infection usually occurs when a person eats food contaminated with the feces of animals or humans carrying the bacteria. Clinical salmonellosis caused by nontyphoidal salmonellae in humans is commonly accompanied by diarrhea, abdominal cramps and fever [17]. Illness has been linked to a wide range of food items including eggs, chicken, beef, pork, salad, vegetables, and dairy products, and other risk factors including overseas travel [18, 19].

Table 1 Foodborne disease-causing pathogens that frequently cause illness worldwide.

Organism	Onset time after ingesting	Signs and symptoms	Duration	Food sources
<i>Escherichia coli</i> producing toxin	1-3 days	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more days	Water or food contaminated with human feces
<i>Escherichia coli</i> O157:H7	1-8 days	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger.	5-10 days	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and vegetables, and contaminated water
<i>Listeria monocytogenes</i>	9-48 hours for gastro-intestinal symptoms, 2-6 weeks for invasive disease	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. The elderly or immunocompromised patients may develop bacteremia or meningitis.	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats
<i>Salmonella</i> spp.	6-48 hours	Diarrhea, fever, abdominal cramps, vomiting	4-7 days	Eggs, poultry meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables
<i>Campylobacter</i> spp.	2-5 days	Diarrhea, cramps, fever, and vomiting; diarrhea may be bloody	2-10 days	Raw and undercooked poultry, unpasteurized milk, contaminated water

2.3 *Listeria monocytogenes*

Listeria monocytogenes is a rod-shaped gram-positive and motile bacterium that is present in different environments. This bacterium is an important human foodborne pathogen and the third leading cause of foodborne deaths due to microbial causes in the USA [20]. *Listeria monocytogenes* is the etiologic agent of listeriosis, an opportunistic, invasive illness that occurs in immunocompromised individuals, such as HIV patients, elderly persons, infants, and pregnant women [21]. Symptoms vary from febrile gastroenteritis and flu-like symptoms to more severe clinical symptoms, such as encephalitis, meningitis, and bacteremia in immunocompromised individuals and spontaneous abortion in pregnant women [22]. It is believed that the main route of *L. monocytogenes* transmission occurs through the consumption of contaminated foods such as raw meats, raw vegetables, ready-to-eat seafood, raw seafood, unpasteurized milk, soft-service ice creams, and soft cheeses [23].

2.4 *Campylobacter* spp.

Campylobacter are of particular research interest as they consistently cause the greatest number of confirmed foodborne bacterial infections in developed countries. The most important species of *Campylobacter* are the thermophilic species: *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*. The majority (over 90%) of the campylobacteriosis are caused by *C. jejuni* and to a lesser extent *C. coli* [24]. The intestinal tract of food-producing animals has been considered as one of the most

important reservoirs for *Campylobacter* in the food supply chain. Poultry are the natural host for *Campylobacter* species and broilers are often colonized, especially with *C. jejuni* [25]. Human exposure can come through consumption of animal products, particularly in raw or undercooked broiler meat [3, 26].

3. Antibacterial activity of plant extracts

Many studies report the antibacterial activity of plant extracts, including activity against bacterial foodborne pathogens. These studies are with different types and parts of plants, such as herbs, spices, leaves, seeds and fruits. A study conducted by Shan et al. [27] evaluated the *in vitro* antibacterial activity of a total of 46 extracts from dietary spices and medicinal herbs against five foodborne pathogens (*Bacillus cereus*, *L. monocytogenes*, *Staphylococcus aureus*, *E. coli* and *Salmonella* serovar Anatum). Gram-positive bacteria were generally more sensitive to the tested extracts than Gram-negative; *S. aureus* was the most sensitive and *E. coli* the least sensitive. The authors reported a highly positive relationship between antibacterial activities and phenolic content of the tested extracts against each bacterium, suggesting that the antibacterial activity was closely associated with their phenolic constituents. On the other hand, Weerakkody et al. [28] studied the antibacterial activity of extracts of less used herbs and spices, and they reported a poor correlation between antibacterial activity against foodborne pathogens (*L. monocytogenes*, *S. aureus*, *E. coli* and *S. Typhimurium*) and phenolic compounds, demonstrating that the antibacterial activity was due to substances other than phenolic compounds.

Mhalla et al. [29] investigated the antibacterial activity of *Rumex tingitanus* leaf extracts and derived fraction and, from their results, they found that the ethyl acetate fraction showed the most potent antibacterial activity. This fraction eradicated the *L. monocytogenes* population in a concentration of 1.25 and 2.5 mg.mL⁻¹ after 20 and 10 minutes of contact time respectively. *Psidium guajava* L. (guava), especially the leaves, possesses useful pharmacological activities. Extracts from leaves of this species showed activity against Gram-positive bacteria (*S. aureus* MIC=100 µg.mL⁻¹) and Gram-negative bacteria (*E. coli* MIC=250 µg.mL⁻¹ and *Pseudomonas aeruginosa* MIC=500 µg.mL⁻¹) [30]. The polyphenols from muscadine grape seed (*Vitis rotundifolia* Michx.) (MIC=54.8-60.1 µg.mL⁻¹) showed stronger inhibition of *S. aureus* than polyphenols from muscadine grape skin (MIC=70.7-80.2 µg.mL⁻¹) [31]. The grape seed extract inhibited the growth of *E. coli* O157:H7 (MIC=4.0 mg.mL⁻¹), and with 0.25-2.0 mg.mL⁻¹ there was a reduction of Shiga toxin production (Stx) without inhibiting the development of the microorganism [32].

Basile et al. [33] assessed the antibacterial activity of the ethanol extract from *Paullinia cupana* Mart. seeds, commonly called guarana. The extract, at a concentration between 16 and 128 µg.mL⁻¹, showed a significant antibacterial effect against both Gram-negative and Gram-positive bacteria, in particular *P. aeruginosa* (MIC=16 µg.mL⁻¹), *E. coli*, *Proteus mirabilis* and *Proteus vulgaris* (MIC=32 µg.mL⁻¹), which were the most inhibited. According to the authors, the antibacterial activity of the extract was probably due to the polyphenols present. Medina et al. [34] evaluated the antibacterial activity of aqueous and acetone extracts of red and yellow araçá (*Psidium cattleianum* Sabine) against *S. Enteritidis*. All araçá extracts showed antibacterial activity with MIC of 5% except for the aqueous extract of red araçá (MIC=16%). Shen et al. [35] examined the antibacterial effect of blueberry (*Vaccinium corymbosum* L.) extracts obtained from four cultivars on the growth of *L. monocytogenes* and *S. Enteritidis*. In general, the extracts at 900 mg.mL⁻¹ exhibited a growth-inhibitory effect against *L. monocytogenes*. The Elliott or Darrow extracts at 900 mg.mL⁻¹ reduced *S. Enteritidis* population to <1 CFU.mL⁻¹. Côté et al. [36] evaluated the antibacterial effect of cranberry (*Vaccinium macrocarpon*) phenolic extract against seven foodborne pathogens. The authors found the MIC values ranged from 12.6 to 50.4 µg phenol/well among tested pathogens using methanol/water (85/15, v/v).

4. Compounds with antibacterial activity

4.1 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids [37]. These compounds are commonly found in the Solanaceae and Fabaceae families [38]. Erdemoglu et al. [39] evaluated the alkaloid profile of the aerial parts of *Lupinus angustifolius* L. and showed antibacterial activity against *Bacillus subtilis*, *S. aureus* and *P. aeruginosa* (MIC=62.5 µg.mL⁻¹). In that study, the capillary GC-MS determined the 13 α -hydroxylupanine (50.78%) and lupanine (23.55%) as the two main alkaloids in the aerial parts of *L. angustifolius*. According to the authors, the presence of lupanine and 13 α -hydroxylupanine explains the antibacterial activity of the extract, as described also by Tyski et al. [40] (Fig. 1). The ability of the some alkaloids to bind tightly to DNA and inhibit topoisomerase II [41] may explain its antibacterial capacity.

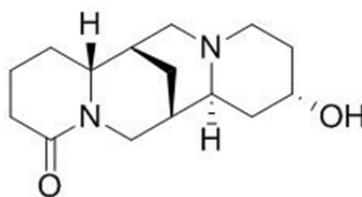


Fig. 1 13-Hydroxylupanine

4.2 Phenolics

Phenolics are one of the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known. Plant phenolics are generally involved in defense against ultraviolet radiation, aggression by pathogens, and contribute to plants color. The phenolic group includes mainly phenolic acids, flavonoids, and tannins [42].

4.2.1 Phenolic acids

Chemically, phenolic acids have at least one aromatic ring in which at least one hydrogen is substituted by a hydroxyl group. These compounds can be divided into two major groups, which are derived from non-phenolic molecules of benzoic acids (hydroxybenzoic acids) and from cinnamic acid (hydroxycinnamic acid) (Fig. 2) [43]. The antibacterial activity of compounds from this group has been reported [44], and variations in the structure of the compounds influence the antibacterial potential of phenolic acids. For example, caffeic acid is more effective than *p*-coumaric acid due to the number of OH groups in the phenolic ring [45]. A possible mechanism to explain the antibacterial action of phenolic acids against pathogens is hyperacidification at the plasma membrane interphase, which alters cell membrane potential, making it more permeable, as well as affecting the sodium and potassium ATPase pump implicated in ATP synthesis [46, 47]. Lou et al. [48] demonstrated that *p*-coumaric acid has dual mechanisms of bactericidal activity: disrupting bacterial cell membranes and binding to bacterial genomic DNA to inhibit cellular functions, ultimately leading to cell death.

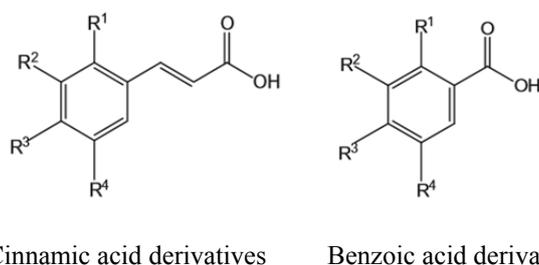


Fig. 2 Chemical structures of cinnamic and benzoic acid derivatives [43]

4.2.2 Flavonoids

Flavonoids are natural pigments abundantly present in the plant kingdom. The position of linkage of the B-ring to the C-ring is used to sort these molecules into three different classes: flavonoids, isoflavonoids and neoflavonoids (Fig. 3).

Other plant secondary metabolites containing a similar carbon skeleton, such as chalcones, stilbenes, or aurones are considered minor flavonoids [49]. Many studies reported the antibacterial activity of flavonoids [50, 51]. Cushnie and Lamb [52, 53] suggested that the antibacterial activity of flavonoids may be attributable to up to three mechanisms: cytoplasmic membrane damage causing perforation and/or reduction in membrane fluidity, inhibition of nucleic acid synthesis caused by topoisomerase inhibition, and inhibition of energy metabolism caused by NADH-cytochrome C reductase inhibition.

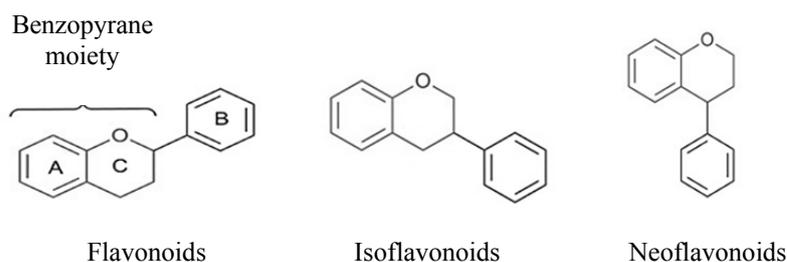


Fig. 3 Chemical structures of flavonoids, isoflavonoids and neoflavonoids [49]

4.2.3 Tannins

Tannins are a complex group of natural polyphenols. Tannins are classified into two major groups on the basis of their structure: the hydrolysable and the condensed tannins. Hydrolysable tannins are compounds containing a central core of glucose or another polyol with gallic acid. Condensed tannins are oligomers or polymers composed of flavan-3-ol nuclei (Fig. 4) [54]. The biological activity of plant extracts containing tannins has been known for centuries, which has led to the isolation and characterization of many representatives of this class [55]. The ability of tannins to inhibit the development of pathogenic microorganisms has been recognized. The different mechanisms proposed so far to explain tannin antimicrobial activity include the inhibition of extracellular microbial enzymes or deprivation of substrates caused by the typical astringent characteristic of tannins, direct action on microbial metabolism through inhibition of oxidative phosphorylation, and a mechanism involving metal ions deprivation [56].

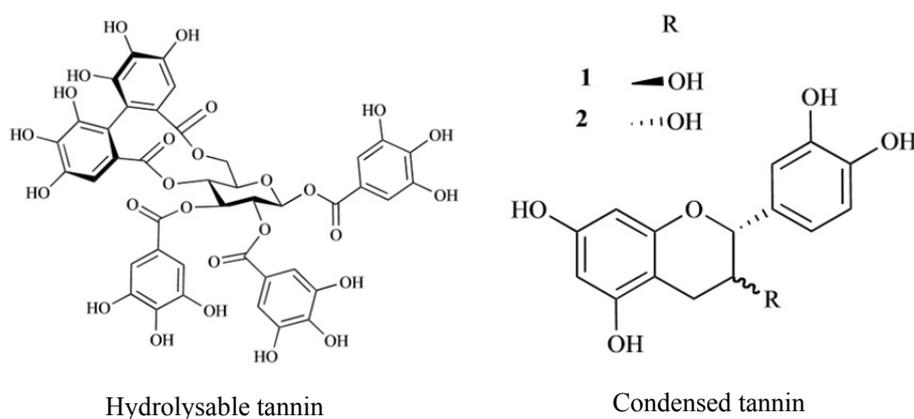


Fig. 4 Example of hydrolysable (tellimagradin II) and condensed (1: catechin; 2: epicatechin) tannin [54]

4.3 Terpenoids

Terpenoids are a diverse group of secondary metabolites, estimated to be around 40,000 compounds (Fig. 5) [57]. Terpenoids are important constituents of essential oils [4]. Several pieces of research reported the antibacterial action of essential oils [58, 59, 60, 61] as well as its mechanism of action [62, 63]. Kurecki et al. [64] tested the antibacterial potential of five terpenoid compounds (α -bisabolol, α -terpinene, cineole, nerolidol and terpinen-4-ol) and proved that terpinen-4-ol showed the highest activity against *Campylobacter* spp. and other reference strains.

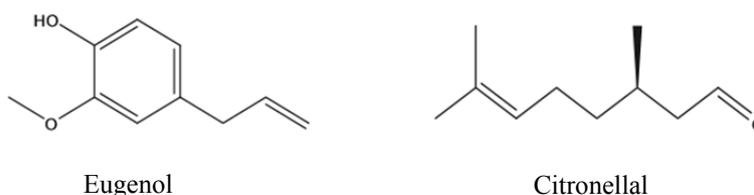


Fig. 5 Example of terpenoids [65]

4.4 Saponins

Saponins are molecules typically formed by an aglycone unit (sapogenin, hydrophobic) attached to a sugar (glycone, hydrophilic) [66]. Liu and Henkel [67] considered the polyphenols and saponins as the key ingredients in remedies used in traditional Chinese medicine responsible for most of the observed biological effects. Saponins have been described as antibacterial compounds, as reported by Hassan et al. [68]. The authors evaluated the antibacterial activity of saponin-rich extract from guar meal and showed antibacterial effect against *S. aureus*, *S. Typhimurium* and *E. coli*, although the mechanism of antibacterial action of saponins is unclear. However, increasing antibiotic activity was observed going from the saponin extracts to the sapogenin samples, suggesting that the sugar moiety is not important for the antimicrobial efficacy [69].

5. Potential food applications of plant extracts

Although many studies report the antibacterial activity of plant extracts *in vitro*, there is some difficulty in replicating the results *in situ*. According to Hayek et al. [7], foods are not sterile, so a mixed microbial population can influence the results that are obtained by *in situ* assays. Miceli et al. [70] evaluated the antibacterial activity of *Borago officinalis* and *Brassica juncea* aqueous extracts *in vitro* and *in situ* using different food model systems (meat broth, fish broth and vegetable broth). The final extract concentration that showed inhibition *in vitro* was not confirmed *in situ*; however, when added at a concentration 10-fold higher than that *in vitro* (100 and 31 mg.mL⁻¹ *B. officinalis* and *B. juncea*, respectively), they inhibited the growth of the sensitive strains. According to the authors, this was not surprising, since the activity of inhibitory substances in foods can be influenced negatively by several factors, such as binding of the active compounds to additives of the food or inactivation by food inhibitors.

Zhang et al. [71] determined the effect of extracts from *Eugenia caryophyllata* and *Rosemarinus officinalis* alone and combined in raw chicken meat during storage for 15 days at 4 °C. The bacterial counts of the chicken samples with a combination of extracts were lower than those of control samples during storage. The antibacterial effect of chestnut inner shell extract was characterized against *C. jejuni* strains on chicken meat [72]. The pathogen was not detected at 1 mg.g⁻¹ of extract with 3 log CFU.g⁻¹ of inoculum after 4 days at 4 °C.

Mariem et al. [73] applied the aqueous extract of the fruits of *Nitraria retusa* in beef patties. In general, there was a significant inhibition of the microbial growth in beef patties containing the fruit extract compared with control meat (without extract of *N. retusa*). The antimicrobial activity of the fruit extract against microbial proliferation was most effective on coliforms. By the end of the storage time (9 days at 4 °C), coliform populations in the control sample were 6.47 log CFU.g⁻¹ and significantly lower by 4.48, 4.13 and 3.81 log CFU.g⁻¹ when the sample was treated with 0.5%, 0.75% and 1% of *N. retusa* extract, respectively.

Plant extracts could be used as natural antimicrobials, but the amount required for microbial inhibition in foods would be considerably high, adversely affecting the sensory characteristics of the food. The use of natural compounds in combination with other natural antimicrobials or with other technologies could produce a synergistic effect against foodborne pathogens [45]. In this context, the application of plant extracts in films can be an alternative to increase the shelf life of foods. Choulitoudi et al. [74], for example, applied extracts and essential oil of *Satureja thymbra* (L.) in edible films to prolong the shelf life of fresh gilthead seabream (*Sparus aurata*) fillets. The ethyl acetate extract alone and combined with essential oil showed the best antimicrobial effect, resulting in 25 and 35% shelf life extension, respectively. Krasniewska et al. [75] determined the antimicrobial properties of pullulan coating enriched with extract from *Bergenia crassifolia* on pepper. After 14 days of storage, the reduction of the *S. aureus* population on pepper coated with pullulan and pullulan with extract was 0.74 and 1 log CFU.g⁻¹, respectively, compared to the uncoated samples.

6. Conclusions

The bioactive properties of plants have long been known. In order to find alternatives to synthetic chemical additives, studies on the antimicrobial effect of plant extracts against foodborne pathogens are being conducted with promising results. In recent years, advances have been made in the study of the action mechanisms of antibacterial compounds found in the extracts. Nowadays, studies are conducted on the application of extracts and/or compounds directly on foods or using antimicrobial films. In these studies there was a decrease in the microbial population of foods. Therefore, the application of plant extracts can be an alternative to synthetic preservatives to increase the shelf life of foods.

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