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In vitro antimicrobial activity of brazilian native fruit pomace extracts and essential oils against multidrug-resistant *Pseudomonas aeruginosa* of animal origin

Atividade antimicrobiana *in vitro* de extratos de frutas nativas brasileiras e óleos essenciais contra isolados multirresistentes de *Pseudomonas aeruginosa* de origem animal

Andryara Panizzon[1], Carla Patricia Freitas[1], Caroline Antunes do Nascimento[1], Lára Franco dos Santos[1], Luana Pasqualotto[1], Milena Zanoello Bertuol[1], Karen Apellanis Borges[2], Thales Quedi Furian[2], Letícia Trevisan Gressler[3,4], Luciana Ruschel dos Santos[1]

1 Universidade de Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brazil
2 Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil
3 Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil
4 Instituto Federal Farroupilha (IFFar), Frederico Westphalen, Rio Grande do Sul, Brazil

*corresponding author:: karen.borges@ufrgs.br

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Abstract: *Pseudomonas aeruginosa* is a multidrug-resistant pathogen of growing concern in both human and veterinary medicine. Its increasing resistance profile, including that of animal-derived strains, necessitates alternative antimicrobial strategies within the One Health approach. This study evaluated the *in vitro* antimicrobial activities of four native Brazilian fruit pomace extracts (guabiroba, uvaia, araçá, and butiá) and four commercial essential oils (cinnamon, clove, ginger, and thyme) against clinical *P. aeruginosa* isolates obtained from domestic animals. Antimicrobial susceptibility and minimum inhibitory concentrations (MICs) were determined using the Kirby–Bauer disk diffusion method and a 96-well plate-based microdilution assay, respectively. The pomace extracts of guabiroba and uvaia showed inhibitory activity against 57.4 % of the tested isolates, whereas araçá and butiá exhibited no detectable effects. Cinnamon oil was the only compound that inhibited all isolates in both assays, with MIC values ranging from 4.88 to 9.70 µg/mL. The clove and thyme oils showed limited activity, whereas the ginger oil was largely ineffective. These findings highlight the potential of cinnamon oil as an antimicrobial candidate and suggest that native fruit residues may represent valuable, sustainable resources. Future studies should focus on investigating the mechanisms of action, synergistic effects, and in vivo efficacy of these compounds to support the development of phytotherapeutic alternatives against multidrug-resistant *P. aeruginosa* strains.

Key-words: antimicrobial action; cinnamon; guabiroba; natural compounds; uvaia.

Resumo: *Pseudomonas aeruginosa* é um patógeno com resistência múltipla aos antimicrobianos e de grande relevância em saúde humana e animal. Seu perfil de multirresistência, observado inclusive em isolados de origem animal, evidencia a necessidade de estratégias alternativas aos antimicrobianos em uma abordagem de Saúde Única. Este estudo avaliou a atividade antimicrobiana *in vitro* de quatro extratos de frutas nativas do Brasil (guabiroba, uvaia, araçá e butiá) e de quatro óleos essenciais comerciais

(canela, cravo, gengibre e tomilho) contra isolados de *P. aeruginosa* obtidos de animais domésticos. A suscetibilidade antimicrobiana foi determinada através do teste de disco-difusão de Kirby-Bauer e da concentração inibitória mínima (CIM) em microplacas de 96 poços. Os extratos de frutas de guabiroba e uvaia demonstraram ação antimicrobiana para 57,4% dos isolados, enquanto araçá e butiá não apresentaram ação antimicrobiana. O óleo de canela foi o único composto a inibir todos os isolados nos dois testes, com valores de CIM entre 4,88 a 9,70 µg/mL. Os óleos de cravo e tomilho apresentaram ação antimicrobiana limitada, enquanto o gengibre foi o menos efetivo. Essas descobertas destacam o potencial do óleo de canela como um promissor candidato antimicrobiano e sugerem que resíduos de frutas nativas podem representar recursos valiosos e sustentáveis. Estudos futuros devem se concentrar na elucidação dos mecanismos de ação, efeitos sinérgicos e eficácia in vivo para subsidiar o desenvolvimento de alternativas fitoterápicas contra cepas resistentes de *P. aeruginosa*.

Palavras-chave: canela; compostos naturais; guabiroba; resistência antimicrobiana; uvaia.

1. Introduction

Pseudomonas aeruginosa is a gram-negative bacterium belonging to the family Pseudomonadaceae. It can survive in various environments, including water and soil, and is an opportunistic pathogen in both humans and animals [1,2]. *P. aeruginosa* is one of the most common microorganisms associated with nosocomial infections, particularly in immunocompromised individuals [3]. Therefore, it is classified among the "ESKAPE" group of nosocomial pathogens, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* species [4].

P. aeruginosa exhibits an intrinsically low susceptibility to several antimicrobials and possesses a remarkable capacity to acquire resistance [4]. Accordingly, it has been listed in the highest-priority group of pathogens by the World Health Organization, urging the development of novel antibiotics [5]. Domestic animals are important reservoirs of *P. aeruginosa* and can directly transmit it to humans via saliva, aerosols, urine, feces, and other forms of close contact [6]. Human strains of *P. aeruginosa* may acquire resistance through horizontal gene transfer or mutational changes from animal isolates [1,6]. However, the role of domestic animals as a source of multidrug-resistant strains is often underestimated [6].

Considering the increase in antimicrobial resistance and consumer concerns regarding the negative effects of synthetic chemicals, natural compounds have garnered interest as alternative antimicrobial agents [7]. Essential oils and plant extracts are considered ecologically sustainable options for controlling pathogenic bacteria. Several natural compounds have shown inhibitory effects against *P. aeruginosa* [8-10].

Brazil harbors a rich biome with numerous native species that can be used to develop novel products; however, these resources are underutilized [11]. The four most common native fruit species in southern Brazil are araçá, butiá, uvaia, and guabiroba [12]. Araçá (*Psidium cattleianum*), also known as araçá-do-mato, araçá-do-campo, or araçá-amarelo, is one of the most economically important species belonging to the Myrtaceae family. It is found over an extensive area of the Brazilian Atlantic coast, from Bahia to Rio Grande do Sul, and extends into northeastern Uruguay [13-15]. Native to southern Brazil and Uruguay, the butiá (*Butia eriospatha*) is a palm fruit of the Arecaceae family, adapted to environments with high levels of abiotic stress [16,17]. Guabiroba (*Campomanesia xanthocarpa*), also known as guavirova, guabirobeira-do-mato, or guabira, is a fruit belonging to the Myrtaceae family, native to Brazil

and distributed across several states [18]. Uvaia (*Eugenia pyriformes*) is a fruit native to the Atlantic Forest, occurring in several Brazilian regions, especially in São Paulo and Rio Grande do Sul [19,20]. These fruits are rich in phenolic compounds, carotenoids, and ascorbic acid [21].

The chemical composition of essential oils is attributed to complex biosynthetic pathways that vary among plant families, genera, and species. Thyme (*Thymus vulgaris* Linn) is an herb in the Lamiaceae family that is native to the western Mediterranean region. The main constituents of thyme oil, which is extracted from the leaves and flowers of the thyme plant, include thymol, γ-terpinene, 1,8-cineole, carvacrol, and linalool [22,23]. Clove (*Eugenia caryophyllata*, family Myrtaceae), with its strong aromatic odor and pungent, distinctive flavor, is cultivated mainly in Madagascar, Tanzania, Sri Lanka, Brazil, and Indonesia. Eugenol is the major component of clove oil [24,25]. Ginger (*Zingiberaceae officinale* Roscoe) belongs to the Zingiberaceae family and is native to Southeast Asia. It is characterized by the presence of essential oils in all parts of the plant, with gingerol as the primary bioactive component [26,27]. Cinnamon (*Cinnamomum cassia*, family Lauraceae) is native to Sri Lanka and other tropical regions of Asia. Its essential oil is composed mainly of cinnamaldehyde, linalool, α-terpinene, and limonene [28,29].

Due to increasing antibiotic resistance and frequent treatment failures, the demand for alternative therapies, such as plant extracts and oils, is growing. In addition, these natural products have been considered for use in pets. However, data on the efficacy of these extracts and oils against *P. aeruginosa* remain scarce.

This study aimed to provide a novel contribution to the literature by evaluating the antimicrobial activity of native Brazilian fruit pomace extracts and essential oils against multidrug-resistant *P. aeruginosa* isolates obtained from domestic animals. Unlike previous studies that have primarily focused on standard strains or human isolates, this approach integrates the One Health perspective with the valorization of agroindustrial residues, offering translational potential for sustainable and alternative antimicrobial strategies in veterinary and potentially human medicine.

2. Material and methods

2.1 *Pseudomonas aeruginosa* strains

Seven *P. aeruginosa* isolates were randomly selected from a stock collection. These strains were originally isolated from domestic animals (horses, dogs, and cats) in northern and central Rio Grande do Sul, Brazil, between November 2022 and May 2023 [30]. A standard strain of *P. aeruginosa* (ATCC 27853) was included.

The isolates were frozen in brain heart infusion broth (Granu Cult, Darmstadt, Germany) supplemented with 20 % glycerol. An aliquot (10 µL) of the stock was inoculated into 4 mL of tryptic soy broth (TSB; Kasvi, Pinhais, Brazil) and incubated at 37 ºC for 24 h. After incubation, the culture was streaked onto cetrimide agar (Granu Cult) and incubated at 37 ºC for 24 h. Colonies were transferred to tubes containing 4 mL of TSB broth, and the turbidity was adjusted to 0.5 on the McFarland scale (1.5×10^8 CFU/mL). The standardized inoculum was then plated onto Mueller–Hinton agar (Kasvi).

2.2 Brazilian native fruits pomace extracts

A total of 20 g of freeze-dried pomace from four Brazilian native fruits was used in this study: guabiroba, araçá, uvaia, and butiá. Extracts were obtained as previously described [11] and the total phenolic content was determined using a spectrophotometer [11].

2.3 Essential oils

Four commercial essential oils were used in this study: cinnamon, clove, ginger, and thyme. These were provided by Oshadi (Colombo, Brazil); their physiochemical properties are described in Table 1.

Table 1. Essential oils: common and botanical names; part of plant; country of origin; main composition; and Chemical Abstract Service (CAS) number.

Common name	Botanical name	Part of the plant	Origin	Main composition	CAS number
Chinese cinnamon	*Cinnamomum cassia*	twigs	China	Cinnamaldehyde (82.3 %) Acetic acid, cinnamyl ester (2.1 %), Coumarin (1.7 %), 2-Propenal, 3-(2)-methoxyphen (13.8 %)	8007-80-5
Clove	*Eugenia caryophyllata*	blossom	Madagascar	Eugenol (84.2 %), eugenyl acetate (10.2 %), β-caryophyllene (4.2 %)	8000-34-8
Ginger	*Zingiber officinalis*	rhizome	India	α-zingiberene (32.1 %), ar-curcumene + β-zingiberene (19.4 %), bisabolene (6.5 %), β-sesquiphellandrene (4.2 %)	8007-08-7
Thymus	*Thymus vulgaris*	blossom, plant	Spain	Thymol (49.1 %), p-cymene (18.1 %)	8007-46-3

2.4 Antimicrobial susceptibility determination

The antimicrobial susceptibility of *P. aeruginosa* was determined using the Kirby–Bauer disk diffusion method, in accordance with the VET08 guidelines of the Clinical and Laboratory Standards Institute [31]. *S. aureus* ATCC 25923 was selected as a control strain to validate the test. The following antibiotic disks (Laborclin; Viamão, Brazil) were tested: amoxicillin (AMX, 10 µg); cefaclor (CEC, 30 µg); ceftriaxone (CRO, 30 µg); cephalexin (CPX, 30 µg); ciprofloxacin (CIP, 5 µg); chloramphenicol (CHL, 30 µg); cephalothin (CEP, 30 µg); cefazolin (CZN, 30 µg); clindamycin (DA, 2 µg); enrofloxacin (ENR, 5 µg); imipenem (IMP, 10 µg); kanamycin (KAN, 30 µg); levofloxacin (LVX, 5 µg); meropenem (MEM, 10 µg); neomycin (NEO, 30µg); ofloxacin (OFX, 5 µg); oxacillin (OX, 1 µg); penicillin G (PEN, 10 µg); streptomycin (STR, 10 µg); and sulfamethoxazole + trimethoprim (SXT, 25 µg). Isolates classified as "intermediate" were considered non-susceptible. Multidrug-resistant (MDR) isolates were defined as those resistant to three or more antimicrobial classes [32].

2.5 Antimicrobial activity assessment of essential oils and fruit pomace extracts using the agar diffusion method

For agar diffusion assays, 100 µL of the bacterial inoculum was spread onto Mueller–Hinton agar. Wells (6 mm) were drilled into the agar, and 40 µL of each extract or essential oil was added to each well. An ofloxacin disk (5 µg) was used as a positive control. Plates were incubated at 37 ºC for 24 h, and inhibition zone diameters were measured using a ruler [11].

2.6 Minimum inhibitory concentration (MIC)

MIC testing was performed in sterile flat-bottom 96-well polystyrene microplates. Each well received 100 µL of Mueller Hilton broth (BD, Grenoble, France). In the first column, 100 µL of extract or oil was added, followed by serial two-fold dilutions across the subsequent columns. Thereafter, 100 µL of the standardized bacterial inoculum was added to each well. The plates were incubated at 36 ºC for 24 h. The assay was performed in triplicate. After incubation, MIC was determined using resazurin (Êxodo Científica, Sumaré, Brazil) at 100 µg/mL as an indicator. An aliquot of 30 µL of resazurin solution was added to each well, and the color change was evaluated after 2 h. Wells that remained blue indicated no bacterial growth, whereas a pink color indicated bacterial multiplication [11].

3. Results

3.1 Antimicrobial susceptibility

The antimicrobial resistance profiles of each isolate are shown in Table 2. All tested isolates were classified as MDR.

Table 2. *Pseudomonas aeruginosa*: antimicrobial resistance profiles.

Isolates	Antimicrobial agent
01	CEC, CHL, CZN, STR, KAN, OX, PEN
02	CEC, CHL, CEP, CZN, STR, KAN, PEN, LVX
03	PEN, SXT, CPX, ENR, AMX, STR, CRO, DA, CEP, CIP
04	IMP, MEN, NEO
05	IMP, MEN, NEO
06	CEC, CHL, CZN, OX, PEN, STR
07	CEC, CHL, CZN, STR, KAN, OX, PEN, LVX, OFX

Amoxicillin (AMX), cefaclor (CEC), ceftriaxone (CRO), cephalexin (CPX), ciprofloxacin (CIP), chloramphenicol (CHL), cephalothin (CEP), cefazolin (CZN), clindamycin (DA), enrofloxacin (ENR), imipenem (IMP, 10 µg), kanamycin (KAN), levofloxacin (LVX), meropenem (MEM), neomycin (NEO), ofloxacin (OFX, oxacillin (OX), penicillin G (PEN), streptomycin (STR), and sulfamethoxazole + trimethoprim (SXT).

3.2 Antimicrobial activity of fruit pomace extracts

Table 3 summarizes the agar diffusion assay results and MIC values for the fruit pomace extracts against *P. aeruginosa*. The agar diffusion assay revealed that araçá and butiá did not inhibit the growth of *P. aeruginosa*. MIC values for araçá ranged from 49.95 to 99.90 mg/mL, whereas those for butiá were 24.97 mg/mL across all isolates. Guabiroba and uvaia exhibited antimicrobial activity against 57.41 % (4/7) of the isolates. In contrast, MIC values for guabiroba and uvaia varied across isolates.

Table 3. Inhibition zone diameter (mm) and minimum inhibitory concentration (MIC; mg/mL) of fruit pomace extracts against *Pseudomonas aeruginosa*.

Isolates	Araçá		Butiá		Guabiroba		Uvaia	
	Diameter	MIC	Diameter	MIC	Diameter	MIC	Diameter	MIC
1	–	49.95	–	24.97	–	49.95	–	49.95
2	–	49.95	–	24.97	15.66	24.97	17.00	12.48
3	–	49.95	–	24.97	–	49.95	–	49.95
4	–	49.95	–	24.97	10.00	12.48	12.66	24.97
5	–	49.95	–	24.97	11.00	49.95	11.00	24.97
6	–	49.95	–	24.97	10.66	49.95	10.66	24.97
7	–	99.90	–	24.97	–	99.90	–	49.95
ATCC[1]	15.66	24.97	23.66	24.97	–	49.95	–	49.95

Not inhibited (–). [1]*Pseudomonas aeruginosa* ATCC 27853.

3.3 Antimicrobial activity of essential oils by agar diffusion and MIC

Table 4 provides the agar diffusion assay results and MIC values for the essential oils against *P. aeruginosa*. Ginger oil did not inhibit *P. aeruginosa* in the agar diffusion assay; however, inhibition was observed for one isolate at 10,000 mg/mL. In the diffusion assay, clove and thyme oils inhibited only one isolate each. MIC values ranged from 39 to 5,000 µg/mL for clove and from 39 to 1,250 µg/mL for thyme. Cinnamon oil inhibited all *P. aeruginosa* isolates in the agar diffusion assay. MIC values for cinnamon oil ranged from 4.88 to 9.70 µg/mL and were significantly ($p < 0.05$) lower than those of the other compounds.

Table 4. Inhibition zone diameter (mm) and minimum inhibitory concentration (MIC; mg/mL) of essential oils against *Pseudomonas aeruginosa*.

Isolates	Cinnamon		Clove		Ginger		Thyme	
	Diameter	MIC	Diameter	MIC	Diameter	MIC	Diameter	MIC
1	31.66	4.88	–	5,000	–	–	–	39
2	26.66	9.70	–	156	–	–	–	156
3	31.66	9.70	–	78	–	–	–	156
4	32.33	9.70	–	5,000	–	–	–	1,250
5	31.00	4.88	–	625	–	–	–	1,250
6	35.00	4.88	–	625	–	10,000	–	312
7	38.66	4.88	11.66	39	–	–	40.00	78
ATCC[1]	31.66	9.7	10	312	–	–	–	625

Not inhibited (–). [1]*Pseudomonas aeruginosa* ATCC 27853.

4. Discussion

P. aeruginosa is a major cause of healthcare-associated infections. In 2017, approximately 32,600 infections occurred in hospitalized patients in the United States, with more than 2,700 attributable deaths [(33)]. Hospital-acquired human pathogens may colonize and infect companion animals [(34)]. Similarly, domestic animals may, in turn, act as reservoirs for *P. aeruginosa*, potentially transmitting it to humans. Thus, controlling *P. aeruginosa* infections is a One Health concern [(6)]. Accordingly, novel strategies,

including essential oils and plant extracts, are required to mitigate the impact of MDR infections on the healthcare system [35]. In this study, we determined the antimicrobial susceptibility profiles of clinical *P. aeruginosa* isolates, all of which were classified as MDR and evaluated the activity of several natural compounds against these isolates.

Waste generation during fruit and vegetable processing is considered a major challenge. However, fruit pomace is rich in phenolics and other bioactive compounds that confer antimicrobial properties [36]. Several fruit pomace extracts have been regarded as potential antimicrobial compounds against pathogenic bacteria [37-39]. Extracts from native fruits hold environmental and economic significance. Therefore, we assessed the antimicrobial activity of several Brazilian native fruit pomace extracts.

The antimicrobial activity of araçá and butiá is largely attributed to the presence of chlorogenic acid, ascorbic acid, and diverse phenolic and carotenoid compounds [37,40]. However, in this study, neither extract inhibited the growth of the *P. aeruginosa* in the agar diffusion method. The MIC values for araçá and butiá extracts were high, reflecting their limited antibacterial activity against *P. aeruginosa*. In contrast, guabiroba and uvaia demonstrated antimicrobial activity against 57.41 % of the isolates. Uvaia is rich in rutin, ellagic acid, gallic acid, and chlorogenic acid, whereas guabiroba is rich in carotenoids and phenolic compounds [37]. Prior research has revealed the antibacterial potential of uvaia against gram-positive bacteria, including *Enterococcus faecalis*, *S. aureus*, and methicillin-resistant *S. aureus* [40-42]. In contrast, gram-negative bacteria, including *Salmonella* Typhimurium and *P. aeruginosa*, appear to be more resistant to the antimicrobial action of uvaia [42-44]. The antimicrobial activity of guabiroba against *P. aeruginosa* has been previously demonstrated [45].

Essential oils can be extracted from several parts of aromatic plants, including the flowers, leaves, seeds, fruits, and roots. They are composed of two or three major components and up to 60 minor constituents [46] The four essential oils selected for this study (cinnamon, clove, ginger, and thyme) previously demonstrated antimicrobial activity against several pathogenic bacteria, including *P. aeruginosa* [47].

In our study, ginger oil did not inhibit *P. aeruginosa* in the agar diffusion assay, and contrary to expectations, only one isolate was inhibited at a high concentration. Previous studies have shown that ginger essential oil can reduce *P. aeruginosa* biofilm formation and virulence [48,49]. However, De Grandis *et al.* [50] reported that the antimicrobial effects of ginger primarily target gram-positive bacteria. In this study, clove and thyme oils exhibited limited activity, as each inhibited only one isolate in the diffusion assay. Cinnamon oil was the only natural compound that inhibited all *P. aeruginosa* isolates in the agar diffusion assay. MIC values for cinnamon oil were significantly lower than those of the other compounds, highlighting its superior potency. Previous studies have demonstrated the broad-spectrum activity of cinnamon oil against both gram-positive and gram-negative bacteria, including MDR *P. aeruginosa* [51,52].

These findings reveal cinnamon essential oil as a standout, exhibiting consistent antimicrobial action against all clinical *P. aeruginosa* isolates and requiring the lowest inhibitory concentrations. Cinnamon oil is well-documented as a broad-spectrum antibacterial agent that damages bacterial cell membranes, alters membrane lipid profiles, and interferes with cellular processes, including ATPase activity, cell division, porin function, and motility. These effects are mainly attributed to its bioactive phytochemicals, especially cinnamaldehyde and eugenol [53]. Given its safety (when used appropriately)

and efficacy against infection-causing bacteria in humans and animals, cinnamon essential oil has emerged as a promising therapeutic candidate. However, clinical trials are lacking, indicating that the clinical efficacy and safety profiles of cinnamon essential oil remain poorly defined [54].

Agar diffusion assays and MIC determination are fundamental for evaluating the antibacterial activities of plant extracts and essential oils. Nevertheless, considerable variability exists in the results reported in the literature. Such differences may be attributed to variations in assay execution, intrinsic characteristics of the bacterial isolates, plant origin, harvest time, extract preparation methods, and specific concentrations tested [50].

5. Conclusion

The antimicrobial activity of Brazilian native fruit pomace extracts remains underexplored and warrants further investigation. Among the essential oils tested, cinnamon oil demonstrated the most potent activity against MDR *P. aeruginosa*, highlighting its potential as an alternative therapeutic agent. These findings contribute to the ongoing screening of natural compounds for their potential application against MDR bacteria. Further studies, including clinical trials, are required to establish the efficacy and safety of these compounds for the treatment of infectious diseases.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

The full dataset supporting the results of this study has been published within this article.

Author contributions

Conceptualization: A. Panizzon, L. R. dos Santos. Data curation: A. Panizzon, L. R. dos Santos. Formal analysis: A. Panizzon, L. R. dos Santos. Project management: L. R. dos Santos. Investigation: A. Panizzon, C. P. Freitas, C. A.do Nascimento, L. F. dos Santos, L. Pasqualotto, M. Z. Bertuol, L. R. dos Santos. Methodology: A. Panizzon, C. P. Freitas, C. A. do Nascimento, L. F. dos Santos, L. Pasqualotto, M. Z. Bertuol, L. R. dos Santos. Validation: A. Panizzon, C. P. Freitas, C. A. do Nascimento, L. F. dos Santos, L. R. dos Santos. Visualization: L. T. Gressler, T. Q. Furian, K. A. Borges. Writing (original draft): A. Panizzon, C. P. Freitas, T. Q. Furian, K. A. Borges. Writing (review and editing): L. T. Gressler, T. Q. Furian, K. A. Borges. Software: L. R. dos Santos. Supervision: L. R. dos Santos.

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