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Research Article

The Chemical Featuring, Toxicity, and Antimicrobial Activity of *Psidium cattleianum* (Myrtaceae) Leaves

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It is known that the phytochemical identification and assessment of biological effects caused by the constituent species *Psidium cattleianum*, which belongs to family Myrtaceae, are poorly held in the literature. The aim of the current study is to investigate the composition of secondary metabolites, the toxicity, and the antimicrobial activity of *P. cattleianum* leaves. The crude ethanolic extract of the plant was obtained through maceration and fractionated with hexane, dichloromethane, and ethyl acetate. The crude ethanol extract and the fractions were subjected to phytochemical screening and tested against the microcrustacean *Artemia salina* for toxicological assessment. Antimicrobial tests with crude ethanol extract and the fractions were carried out through the agar diffusion method using broth microdilution against *Staphylococcus aureus*, *S. epidermidis*, *Burkholderia cepacia,* and *Escherichia coli* strains. A variety of secondary metabolite groups such as catechins, steroids, phenolic compounds, flavonoids, and saponins was detected. Regarding toxicity, hexane and dichloromethane fractions were considered nontoxic, whereas the crude ethanol extract and the ethyl acetate fraction showed low toxicity. The crude ethanol extract and the fractions, except for the hexane fraction, showed activity against the tested strains. Therefore, the composition of the secondary metabolites, the low toxicity, and the antimicrobial activity suggest that this species is promising in the search and development of new drugs.

1. Introduction

The human practice of consuming and using natural products to treat, heal, and prevent diseases is ancient and universal. The use of such substances is as old as the history of mankind and plays an important role in the access to basic health care [1, 2].

Traditionally, natural products have been the largest source of new drugs [3, 4]. Over the years, several molecules found in plants that have pharmacological properties allowed discovering remarkable therapeutic innovations to treat different diseases, such as asthma, cancer, hypertension, parasitic diseases, cardiovascular diseases, and microbial infections, and this has significantly improved the quality of life of the population [5–9].

The family Myrtaceae stands out among the several plant families studied in the chemistry of natural products. It is one of the most important plant families found in Brazil due to the large number of fruit species or to pharmacological properties such as analgesic, antimicrobial, antiallergic, antiplasmodial, antioxidant, antidiarrheic, and diabetes control action [10–16]. This family comprises the *Psidium cattleianum* species, which produces a wild fruit popularly known in Brazil as strawberry guava.

Native *Psidium* species (*Psidium* spp.) have attracted great interest of scientific studies in recent years, since their fruits, which are much appreciated for their sensory attributes, show nutritional potential and are rich in vitamins and antioxidants. Such substances show potential in the food and

pharmaceutical industries [17, 18]. The folk medicine uses *Psidium* species for antiseptic purposes: in the treatment of digestive disorders, for antihemorrhagic action, to control blood pressure, as diuretic, and in decoctions in the treatment of diarrhea [19–21].

Some studies have performed the physical, chemical, and antioxidant featuring of guava fruits [22–25]. However, there are few phytochemical-scope studies about the *Psidium cattleianum* [15] species. It is worth mentioning the study by Alvarenga et al. [15], who analyzed the presence of secondary metabolites in *P. cattleianum* leaves and corroborated the presence of flavonoids, saponins, cardiac glycosides, anthraquinones, and tannins in the phytochemical screening.

Similarly, studies addressing the antimicrobial potential of *P. cattleianum* plant extracts are scarce. Medina et al. [19] investigated the antimicrobial potential of *P. cattleianum* fruit extracts (yellow and red fruit varieties) in different solvents against *Salmonella enteritidis* (ATCC 13076). Such microorganism was sensitive to *P. cattleianum* extracts. Brighenti et al. [26] investigated the effect of the aqueous extract from *P. cattleianum* leaves (red fruit variety) on the *Streptococcus mutans* biofilm viability. They found that the extract had antimicrobial activity against the biofilm whenever it was used at high concentrations.

Despite these few studies, the literature has not yet shown studies using *in vitro* toxicity methods that allow comparing and assessing *P. cattleianum* extracts. The small number of studies focused on the composition of secondary metabolites and on assessing the biological effects of *P. cattleianum* leaf constituents highlighting the need to expand scientific studies of this nature, in order to help discovering new biologically active compounds with low toxicological potential. Thus, the aim of the current study is to investigate the composition of secondary metabolites, as well as the toxicity and the antimicrobial activity of *P. cattleianum* leaf constituents (Myrtaceae) (yellow fruit variety).

2. Materials and Methods

2.1. Botanical Material Collection, Identification, and Record. The *P. cattleianum* leaves were collected in a residential backyard located in Pires do Rio County (Goiás State, Brazil). The taxonomic identification was carried out in the Herbarium of the Exact Sciences and Technology Campus at State University of Goiás (CCET/UEG), Anápolis County, Goiás State, Brazil, and registered under number 9203.

2.2. Obtaining the Ethanol Extract and the Fractions. The dried leaves (547,771 g) were crushed and macerated in 95% absolute ethanol (500 mL) for 7 days, at room temperature. The ethanol extract (33.3 g) was dissolved in methanol and water (8 : 2), after solvent removal. Subsequently, it was partitioned with hexane (hexane fraction, 4.1 g), dichloromethane (dichloromethane fraction, 7.2 g), and ethyl acetate (ethyl acetate fraction, 10.6 g). All the samples were concentrated using rotary evaporator under reduced pressure and kept under refrigeration in hermetically sealed vials, until the time they were used in the tests.

2.3. Phytochemical Prospection. The ethanol extract and the fractions were subjected to phytochemical tests, according to the methodology described by Costa [27]. Tests were performed to verify the presence of the following classes of secondary metabolites: (i) saponins (persistent foam); (ii) phenolic compounds (reaction to ferric chloride); (iii) flavonoids (Shinoda reaction); (iv) tannins (reaction to gelatin); (v) steroids (Kedde reaction); (vi) catechins (reaction to hydrochloric acid); (vii) coumarins (reaction to NaOH); (viii) alkaloids (Dragendorff's reagent); and (ix) polysaccharides (reaction to Lugol).

2.4. Toxicity Test on Artemia salina. The test was performed according to the procedure described by Molinas-Salinas and Said-Fernández [28], with modifications. Sixty milligrams (60 mg) of *Artemia salina* cysts was incubated in a container containing synthetic seawater medium prepared with dissolved sea salt (40 $g \cdot L^{-1}$) and supplemented with yeast extract (6 $g \cdot L^{-1}$). The medium was kept under constant oxygen saturation for 48 hours, at room temperature and under natural light, in order to hatch the cysts.

Toxicity was tested on 96-well polystyrene microplates at *P. cattleianum* ethanolic extract concentrations of 2400, 600, 150, 37.5, and 9.3 $\mu g \cdot mL^{-1}$. The bioassays were performed in triplicate and they were accompanied by negative (using synthetic seawater alone) and positive (using potassium dichromate solution, $K_2Cr_2O_7$) controls at concentrations of 100, 50, 25, and 12.5 $\mu g \cdot mL^{-1}$. The median lethal dose (LD_{50}) was calculated according to the Probit method in the StatPlus 2009 software, at 95% confidence.

The toxicity was classified according to the criterion by Nguta et al. [29], who consider LD_{50} up to 100 $\mu g \cdot mL^{-1}$ as high toxicity values, LD_{50} between 100 and 500 $\mu g \cdot mL^{-1}$ as moderate toxicity values, LD_{50} between 500 and 1000 $\mu g \cdot mL^{-1}$ as low toxicity values, and LD_{50} above 1000 $\mu g \cdot mL^{-1}$ as nontoxic values.

2.5. Assessing the Antimicrobial Activity. The agar diffusion (disc diffusion and well diffusion) and the broth microdilution methods were used to assess the antimicrobial activity of the ethanol extract, as well as that of the fractions, as it was recommended by the Clinical and Laboratory Standards Institute (CLSI) [30].

The tests were used against standard strains (ATCC, American Type Culture Collection) derived from the stock culture collection of the CCET/UEG Microbiology Laboratory, Anápolis County, Goiás State, Brazil. Four bacterial strains were used, namely, two gram-positive bacteria, *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidis* (ATCC 12228), and two gram-negative bacteria, *Burkholderia cepacia* (ATCC 17759) and *Escherichia coli* (ATCC 25312).

The disc diffusion test was performed according to the CLSI [30] recommendations, with modifications. Microorganism suspensions were adjusted to 0.5 in the McFarland scale and seeded on Muller Hinton agar surface. Subsequently, filter paper discs (Whatman, type 3), both sterile and impregnated with 10 μL of the tested compounds at the

Table 1: Classes of chemical substances found in the ethanol extract and in the fractions.

Group of metabolites	Ethanol extract	Fraction		
		Hexane	Dichloromethane	Ethyl acetate
Saponin	+	−	−	−
Phenolic compounds	+	−	+	+
Flavonoids	+	−	+	+
Tannins	+	−	+	+
Steroids	+	+	+	−
Catechins	+	+	−	−
Coumarin	−	−	−	−
Alkaloids	−	−	−	−
Polysaccharides	−	−	−	−

+: present; −: undetected.

concentration of 100 mg·mL^{-1}, were distributed in plates. The diameter of the halo (inhibition zone) formed around the discs was read after 24 incubation hours. The antibiotic ampicillin (30 μg/disc) was used as inhibition control.

The well diffusion test was also performed according to the CLSI [30] methodology, with modifications. Approximately, 30 mL of Mueller Hinton agar was spaced in a 15 cm diameter sterile Petri dish, thus resulting in a thin layer on the dish base. The medium became solidified and 10 mL Mueller Hinton agar molten at approximately 45°C with 10% bacterial inoculum standardized to 0.5 of the McFarland scale was poured into the dish containing the solidified agar layer. The new medium became solidified and 6 mm wells were made using the base of 100 μL sterilized tips. A micropipette was used to add 50 μL of the substances to be tested. The antibiotic chloramphenicol (50 μg·mL^{-1}, 10 μL) was used as inhibition control. The plates were incubated at 35°C for 24 hours and, subsequently, the inhibition zone was measured using a millimeter ruler. Student's t-test at 5% significance level was used to compare the values obtained through the agar diffusion techniques (disc diffusion and well diffusion).

The Minimal Inhibitory Concentrations (MIC) of the ethanol extract and its fractions were set through the broth microdilution technique, according to the CLSI [30] protocol, with modifications. In addition, resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) was used as bacterial viability indicator. In short, the tests were performed on Muller Hinton broth contained in a 96-well round bottom microplate. The extract and the fractions were diluted in the broth at the concentrations of 4.8, 2.4, 1.2, 0.6, and 0.3 mg·mL^{-1}. One hundred milliliters (100 mL) of Muller Hinton broth containing the plant extract at the tested concentrations and 10 μL microorganism suspension adjusted to 0.5 of the McFarland scale were placed in each microplate well.

One well was added with 100 μL Muller Hinton broth with 5% DMSO in order to control the solvent. Another well was added with 100 mL plant extract diluted in Muller Hinton broth without bacterial inoculum and it was used as negative control of extract and medium sterility. The plates were covered and incubated in bacteriological incubator at 35°C, for 24 hours.

After the incubation, the resazurin was added to both the test and the control wells in order to identify bacterial growth. The lowest concentration able to inhibit microbial growth was visually determined by observing the resazurin color change after additional incubation for 2 hours: blue, without growth, and pinky, with growth. All the tests were performed in independent triplicate [30].

3. Results and Discussion

3.1. Phytochemical Prospection. Table 1 shows the results of the phytochemical tests performed in *P. cattleianum* leaves.

The results indicate the presence of important classes of secondary metabolites, which account for several therapeutic properties. The saponins, for instance, have been reported to have anti-inflammatory, sedative, expectorant, diuretic, and analgesic properties and they are widely used as adjuvant in oral and injectable vaccines [31, 32]; the phenolic compounds have been reported to have antioxidant, antiulcerogenic, anti-inflammatory, and anticancer properties [33, 34]. The tannins have been used as antidiarrheic, antiseptic, antimicrobial, healing, and anti-inflammatory substances [35]. The catechins have strong antioxidant action. They inhibit the development of tumors, reduce the body fat, and protect the body against diabetes, heart diseases, and inflammations [36].

The plant sterols stand out for reducing the uptake of low-density lipoprotein cholesterol (LDL-C) levels. Consequently, they prevent the development of coronary heart diseases. In addition, plant sterols have the potential to balance the immune system, to alleviate the causes of autoimmune diseases, and to inhibit the growth of certain types of malignant tumors [37].

Phytochemical investigations of several plants of economic importance are reported in several species of the family Myrtaceae. Silva et al. [38] developed a phytochemical study on *Psidium guajava* (Guava) and reported the presence of chemical constituents such as tannins, flavonoids, saponins, and triterpenoids.

Souza [39] conducted a phytochemical study on *Myrciaria cauliflora* (Brazilian grape tree). The author assessed the composition of the classes of secondary metabolites and indicated the presence of flavonoids and tannins. Paula et al. [40] conducted a phytochemical study on *Pimenta pseudocaryophyllus* leaves and detected the presence of phenolic compounds, tannins, and flavonoids. Fiuza et al. [41]

Table 2: Ethanol extract toxicity and different fractions expressed as LD_{50} for *Artemia salina* larvae.

Extract and fractions	LD_{50} $\mu g \cdot mL^{-1}$	Toxicity classification*
Ethanol extract	820.83	Low toxicity
Hexane fraction	>1000	Nontoxic
Dichloromethane fraction	>1000	Nontoxic
Ethyl acetate fraction	272.36	Moderate toxicity
$K_2Cr_2O_7$	14.89	High toxicity

*Classification based on Nguta et al. [29].

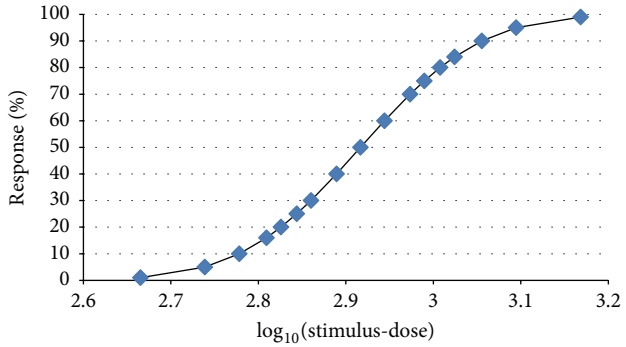


Figure 1: Dose-response effect of the ethanolic extract against *A. salina*.

performed the phytochemical screening of *Eugenia uniflora* (Brazilian cherry) leaves and found steroids, flavonoids, saponins, and tannins.

The phytochemical studies conducted on species belonging to the family Myrtaceae also revealed the absence of alkaloids and coumarins, which is a fact that corroborates the results found in *P. cattleianum* species. Such results indicate that these metabolites are probably absent in the chemical composition of this botanical family [38, 41].

3.2. Assessing the Toxicity. Table 2 shows the results of the *Artemia salina* toxicity tests.

According to the reference values set by Nguta et al. [29], the ethanol extract showed low toxicity, the ethyl acetate fraction showed moderate toxicity, and the hexane and dichloromethane fractions showed no toxic effects; thus, they can be well tolerated by the biological systems.

The dose-response ratio obtained through the logarithm of concentration versus mean percentage of microcrustacean mortality is shown in Figures 1 (ethanolic extract) and 2 (ethyl acetate fraction).

The toxic effect on *A. salina* has shown good correlation with several biological properties in compounds with $LD_{50} < 1000\ \mu g\ mL^{-1}$, such as antifungal [42], antimicrobial [42, 43], antitumor [44–46], and trypanocidal action [47, 48].

Studies conducted with Myrtaceae species reported the toxicity of the hydroalcoholic extract from *Psidium guajava* (LD_{50} = 880 $\mu g \cdot mL^{-1}$), *Syzygium cumini* (LD_{50} = 475 $\mu g \cdot mL^{-1}$), and *Syzygium aromaticum* (LD_{50} = 20 $\mu g \cdot mL^{-1}$) leaves [49], as well as the toxicity of the essential oil from *Myrcia myrtifolia* (LD_{50} = 479 $\mu g \cdot mL^{-1}$) [50] and of the crude extract from *Calycorectes psidiiflorus* [51] leaves (LD_{50} = 186.64 $\mu g \cdot mL^{-1}$) and branches (LD_{50} = 566.24 $\mu g \cdot mL^{-1}$) on *A. salina*. On the other hand, the ethanolic extract from *Eugenia uniflora* [52] leaves showed no toxicity on *A. salina*. The survival of 100% *Artemia salina* in the viability control and the high potassium dichromate toxicity (LD_{50} = 14.89 $\mu g \cdot mL^{-1}$) validate the adequacy of the experimental conditions adopted in the current study.

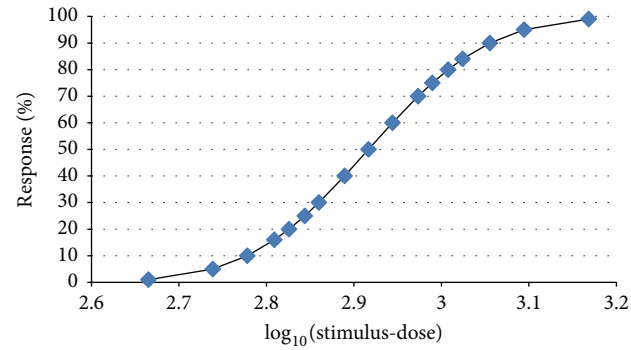


Figure 2: Dose-response effect of the ethyl acetate fraction against *A. salina*.

3.3. Antimicrobial Activity. Table 3 shows the diameters of the growth inhibition zones caused by the substances tested against the bacterial strains.

According to Student's *t*-test, the studied microorganisms were more sensitive to the well diffusion method than to the disc diffusion method. Such fact shows similarity to the study conducted by Silveira et al. [53], who found that the diameter of the halo formed by the well diffusion technique was greater than that obtained by disc technique, probably due to the higher volume of the compounds used in the first technique.

No bacterial growth inhibition zone was formed in the hexane fraction in any of the agar diffusion methods used in the current study. It is worth highlighting that the absence of antimicrobial activity in the hexane fraction may be related to (i) the resistance or insensitivity of the herein studied microorganisms to the action of chemical components found in this fraction or (ii) the difficult penetration and uniform diffusion of the nonpolar molecules found in the hexane fraction throughout the culture medium.

According to the disc diffusion tests, just the ethanol extract was active for two standard strains (*Staphylococcus aureus* and *Staphylococcus epidermidis*) with 9 mm and 11 mm inhibition zones, respectively, and it was considered partially active, according to the classification by De Almeida Alves et al. [48].

As for the well diffusion method, the substances, except for the hexane fraction, showed antimicrobial activity against all the tested bacteria and the diameter of the inhibition zones formed by the ethanol extract (classified as very active) was slightly higher than that formed by the fractions (classified as active).

In addition, *Staphylococcus aureus* and *Staphylococcus epidermidis* were more sensitive to the ethanol extract (the

Table 3: Antibacterial activity of the ethanol extract and of the fractions from *P. cattleianum* leaves against the studied bacterial strains.

Strains	Inhibition zone diameter (mm)											
	Ethanol extract		Fraction						Control			
			Hexane		Dichloromethane		Ethyl acetate		Neg. control		Pos. control	
	DD	WD	DD	DP	DD	WD	DD	WD	DD	WD	DD	WD
S. aureus	9.0 ± 0.8	25 ± 2.0*	—	—	—	14 ± 0.8	—	16 ± 0.4	—	—	32 ± 2.6	12 ± 0.8
S. epidermidis	11 ± 1.4	25 ± 2.4*	—	—	—	18 ± 1.6	—	16 ± 1.2	—	—	15 ± 1.8	25 ± 1.6*
B. cepacia	—	20 ± 0.8	—	—	—	8.0 ± 1.6	—	8.0 ± 1.4	—	—	15 ± 1.6	22 ± 1.4
E. coli	—	22 ± 1.6	—	—	—	16 ± 1.2	—	14 ± 0.8	—	—	23 ± 2.2	21 ± 0.8

DD: disc diffusion. WD: well diffusion. The symbol — indicates no inhibition zone formation.
Inhibition degrees: inactive (<9 mm inhibition zone), partially active (9–12 mm inhibition zone), active (12–18 mm inhibition zone), and active (>18 mm inhibition zone) [48]. The data are expressed as mean ± standard deviation. ∗ indicates statistical difference between the disc and well diffusion technique data, according to Student's *t*-test at 5% probability.

Table 4: Minimum Inhibitory Concentration of the ethanol extract and of its fractions against the studied bacterial strains.

Extract and fractions	MIC (mg mL^{-1})			
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Burkholderia cepacia*	*Escherichia coli*
Ethanol extract	2.4	1.2	4.8	>4.8
Hexane fraction	>4.8	>4.8	>4.8	>4.8
Dichloromethane fraction	>4.8	4.8	>4.8	4.8
Ethyl acetate fraction	4.8	4.8	4.8	1.2

mean diameter of the inhibition zones was 25 mm) and they were followed by *Escherichia coli* (22 mm) and *Burkholderia cepacia* (20 mm).

There are different mechanisms through which secondary metabolites with antimicrobial properties exert action, namely, (i) the disruption or disintegration of cell structures and membranes, (ii) the microbial DNA destruction or inactivation, which inhibits the DNA transcription into messenger RNA as well as the protein synthesis, and (iii) the destabilization of the driving force of protons, the electrons flow, and the selective permeability of the cell membrane [54–56].

Table 4 presents the MIC values of the ethanol extract and that of the fractions.

The studies conducted with the partitions found that the hexane fraction did not inhibit the growth of microorganisms at the studied concentrations, and it may be related to the chemical profile (major compounds) of such fraction. The dichloromethane fraction inhibited the growth of *Staphylococcus aureus* at the concentration of 4.8 mg·mL^{-1}. The ethyl acetate fraction inhibited the growth of *Burkholderia cepacia*, *Staphylococcus aureus,* and *Staphylococcus epidermidis* at the concentration of 4.8 mg·mL^{-1} and *Escherichia coli* at the concentration of 1.2 mg·mL^{-1}. The solvent control (5% DMSO) showed no bacterial growth inhibition.

The antimicrobial activity found in the current study may be associated with the presence of saponins, flavonoids, tannins, and other mixtures in the chemical composition of the studied species, since the literature describes proven antimicrobial activities of these compound classes [2].

Several studies depict the antibacterial activity of genus *Psidium,* family Myrtaceae, such as *Psidium guajava* [10, 57–59], *Myrciaria cauliflora* [60, 61], and *Eugenia uniflora* [62].

Carvalho et al. [57] used the paper disc diffusion method to analyze the antimicrobial activity of hydroalcoholic extracts from *Psidium guajava* leaf and stem on gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp., *Proteus* spp., *Klebsiella* spp., and *Salmonella* spp.). Such microorganisms, except for *Klebsiella* spp., were sensitive to the extracts.

The antimicrobial activity of the hydroalcoholic extract from *Eugenia uniflora* leaves, which was assessed through MIC using the liquid culture medium microdilution method, was checked by Auricchio et al. [62]. It was observed that the extract is able to inhibit the microbial growth in all tested microorganism strains (*Staphylococcus aureus*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans,* and *Aspergillus niger*).

4. Conclusion

The study of the phytochemical composition of *P. cattleianum* showed the presence of catechins, steroids, phenolic compounds, flavonoids, and saponins as secondary compounds. The chemical constituents found in the leaves of this botanical species showed therapeutic applicability potential. Thus, this plant should be investigated in further pharmacological studies. In addition, the popular use of this plant may be related to the results found in the phytochemical assessment. It is worth emphasizing that the chemical composition of the plant leaf is similar to that of other species in the family Myrtaceae.

The constituents of *P. cattleianum* leaves showed low or no toxicity. The ethanol extract and its fractions, which were obtained from the leaves, showed antimicrobial activity and the action spectrum was linked to the significant presence of metabolites with known antibacterial activity, such as

phenolic compounds, saponins, and tannins. Therefore, the results of the current study helped in better understanding the chemical and biological features of the plant species as well as encouraging further studies aimed at the purification, isolation, and structural elucidation of the bioactive components responsible for the antibacterial activity found in *P. cattleianum*.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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