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Phytochemical Analysis, Antioxidant and Antimicrobial Potential of *Pouteria campechiana* (Kunth) Leaf Extracts

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Abstract

Aims: This study investigated the phytochemical composition, antioxidant activity, and antimicrobial potential of the leaves from *Pouteria campechiana* (*Sapotaceae*), a fruit species known for its use in traditional medicine. **Methodology:** Aqueous and ethanolic extracts were prepared using established methods and then subjected to qualitative phytochemical screening and quantification of secondary metabolites. Biological activities were assessed by the DPPH method for antioxidant activity and by liquid diffusion for antimicrobial activity using reference, food, and clinical strains. **Results:** Phytochemical screening revealed the presence of tannins, flavonoids, anthocyanins, reducing compounds, and mucilage. The ethanolic extract proved particularly rich in polyphenols, flavonoids, and condensed tannins, with levels approximately twice those of the aqueous extract. The evaluation of antioxidant activity showed that the ethanolic extract exhibits significant free radical scavenging capacity (IC_{50} = 56.9 µg/mL), superior to that of the aqueous extract (IC_{50} = 326.3 µg/mL). Although ascorbic acid remains more potent, the performance of the ethanolic extract demonstrates the central role of polyphenols in the antioxidant activity of *P. campechiana*. Antimicrobial analyses showed that the aqueous extract was more active in diffusion on agar, with inhibition zones of 8.5 to 14.5 mm against several strains, including *Staphylococcus aureus*, *Listeria*

monocytogenes, and *Escherichia coli*. The Minimum Inhibitory Concentrations obtained (10 to 40 mg/mL) and the ratio Minimum Bactericidal Concentration MBC/MIC greater than 4 indicate a predominantly bacteriostatic effect for all microorganisms. Notably, multidrug-resistant clinical strains were less susceptible to the crude extracts. **Conclusion:** *Pouteria campechiana* appears to be a plant of interest, possessing good antioxidant activity and moderate antimicrobial potential. The ethanolic extract is a promising candidate for antioxidant applications, while the aqueous extract shows interesting antimicrobial efficacy.

Keywords

Phytochemistry, *Sapotaceae*, Biological Activities, Traditional Medicine, Antibiotics, Benin

1. Introduction

Pouteria campechiana (Kunth), commonly called canistel or egg-fruit because of the mealy, yellow texture of its fruit resembling egg yolk, is a fruit species belonging to the *Sapotaceae* family. Native to Central and South America, the species has spread widely in tropical and subtropical regions where it is cultivated for both its fruit and its traditional uses [1]. In West Africa, particularly in Benin, canistel is attracting increasing interest due to its nutritional value, pharmacological properties, and economic potential [2].

The fruit of *Pouteria campechiana* is particularly rich in carotenoids, fiber, vitamins, natural sugars, and phenolic compounds, making it a functional food of interest for human nutrition [3]. Beyond its nutritional value, several parts of the plant, such as the leaves, bark, seeds, and fruit, are used in traditional medicine to treat various ailments such as respiratory conditions, digestive disorders, inflammation, and microbial infections [4]. These empirical uses are attributed to the presence of bioactive secondary metabolites such as tannins, flavonoids, saponins, anthocyanins, and reducing compounds, which are known for their antioxidant and antimicrobial properties [5].

In recent years, *Pouteria campechiana* has attracted the attention of researchers due to its potential in pharmacognosy, agri-food, and biotechnology. Several recent studies have reported antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, justifying its exploration as a source of bioactive natural molecules [3] [6].

However, despite these advances, scientific knowledge remains limited, particularly regarding the biological properties of leaf extracts and their potential against resistant microorganisms. Therefore, studying the phytochemical characteristics and biological activities of *Pouteria campechiana* appears essential to valorizing this plant resource, better understanding its mechanisms of action, and exploring its potential applications in the food, therapeutic, and pharmaceutical fields [2].

Despite the traditional uses of *Pouteria campechiana* leaves, recent scientific data on their phytochemical composition, antioxidant activity, and antimicrobial properties remain insufficient. At the same time, the rapid increase in microbial resistance constitutes a global emergency, necessitating the search for new natural sources of active ingredients [7].

In this context, a question arises: Do the leaves of *Pouteria campechiana* possess a phytochemical richness capable of explaining significant antioxidant and antimicrobial activity, and to what extent do aqueous and ethanolic extracts differ in their biological efficacy against reference, foodborne, and clinical microorganisms?

In other words, which secondary metabolites are present in the leaves?

Are these compounds extracted differently depending on the solvent used?

Which extract exhibits the best antioxidant activity?

Are the extracts active against the tested bacteria and yeasts?

Do foodborne and clinical resistant strains respond to them?

2. Methodology

2.1. Phytochemical Screening

Phytochemical screening was performed on *Pouteria campechiana* powder according to the method of Houghton [8]. It is based on differential precipitation and colorimetric reactions, complemented and improved.

2.2. Extract Preparation

2.2.1. Preparation of the Aqueous Extract

The aqueous extract is a decoction prepared from *Pouteria campechiana* leaf powder. Fifty grams of powder are boiled for 30 minutes in 500 mL of distilled water. After cooling, the resulting decoction is filtered and then evaporated under reduced pressure at 60˚C using a Stuart Rotavapor.

2.2.2. Preparation of the Ethanolic Extract

The ethanolic extract is a maceration of the plant material at room temperature. It was prepared by extracting 50 g of *P. campechiana* leaf powder with 500 mL of ethanol. The resulting macerate is filtered and then evaporated under reduced pressure at 60˚C using a Stuart Rotavapor.

2.3. Assay of Phenolic Compounds

2.3.1. Assay of Total Polyphenols

For the determination of phenolic compounds, the extracts were at a concentration of 20 mg/mL. The determination of total polyphenols was performed according to the method described by Singleton [9]. 200 µL of each extract or of the standard (gallic acid) was taken and dissolved in 1000 µL of 10% Folin-Ciocalteu reagent (FCR). After incubation for 5 min, 800 µL of 75 mg/mL sodium carbonate (Na_2CO_3) was added. The vortexed mixture was incubated for 2 hours. Absorbance readings were taken using a 722 G Visible Spectrophotometer at 760 nm. The

determination was repeated three times. The total polyphenol content is deduced from calibration ranges established with gallic acid (0 - 200 µg/mL) and is expressed in mg of gallic acid equivalent per gram of extract (µg GAE/mg of extract).

2.3.2. Flavonoid Assay

The total flavonoid content of aqueous and ethanolic extracts of *Pouteria campechiana* leaves was determined using the Dowd method modified by [10] with aluminum trichloride ($AlCl_3$). Rutin was used as a reference compound to establish the calibration curve. 1000 µL of 2% $AlCl_3$ solution was taken, and 1000 µL of the sample was added to the test tubes. The blank consisted of 1000 µL of ethanol and 1000 µL of $AlCl_3$. Absorbance readings were taken using a 722 G Visible Spectrophotometer at 415 nm. The assay was repeated three times. The flavonoid content of the aqueous and ethanolic extracts is deduced from calibration ranges established with Rutin (0 - 200 µg/mL) and is expressed in mg of Rutin equivalent per gram of extract (µg RUTq/mg of extract).

2.3.3. Condensed Tannin Assay

The condensed tannin assay was performed according to the method described by Naczk [11]. To 500 µL of each sample or standard, 1000 µL of a 4% vanillin sulfuric acid solution in ethanol (EtOH) was added. The mixture was incubated for 15 min, and the absorbance was read at 500 nm. Condensed tannin concentrations were deduced from calibration ranges established with pyrogallol (0 - 200 µg/mL) and are expressed as µg pyrogallol equivalent per milligram of extract.

2.4. Antioxidant Activity: Determination of Inhibitory Concentration

For this test, samples were prepared in ethanol [12]. For each extract, a 5 mg/mL stock solution is prepared. This solution is then diluted in a geometric series with a ratio of 2 to obtain different concentrations. In dry, sterile test tubes, 1 mL of the solution of the extract to be tested is introduced, followed by 1 mL of DPPH solution (100 µg/mL). After vortexing, the tubes are incubated in the dark at room temperature for 30 minutes. Absorbance is measured at 517 nm using a 722 G Visible Spectrophotometer. For each dilution, a blank is prepared, consisting of 1 mL of the test solution plus 1 mL of ethanol. The positive control is represented by ascorbic acid (100 µg/mL) and is treated under the same conditions as the test sample.

2.5. Exploration of the Antimicrobial Potential of *P. campechiana* Extracts

The exploration of the antimicrobial potential of *P. campechiana* leaf consisted, firstly, of performing sensitivity tests on the extracts (aqueous and ethanolic) against five (5) different reference strains and ten (10) *E. coli* strains, five (5) of foodborne origin and five (5) of clinical origin. Secondly, the antibacterial param-

eters, namely the Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentrations (MBCs), were determined.

2.5.1. Microbial Strains

Fifteen (15) microbial strains were tested in this study. These strains include five (5) reference strains (*Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 19114, *Escherichia coli* ATCC 25922, *Candida albicans* MHMR, and *Pseudomonas aeruginosa* ATCC 27853), five (5) foodborne multidrug-resistant *E. coli* strains (isolated from traditional Tchakpalo beer), and five (5) clinically sourced multidrug-resistant *E. coli* strains (uropathogenic). The reference and clinical strains were provided by the Laboratory of Biology and Molecular Typing in Microbiology (University of Abomey-Calavi, Benin). The foodborne strains were provided by the Laboratory of Microbiology, Food Technology, and Phytopathology (University of Abomey-Calavi, Benin).

2.5.2. Susceptibility Testing

The susceptibility of the microbial strains under study to extracts of *P. campechiana* leaves was evaluated using the solid-media diffusion method described by Benguesmia and Chellouf [13]. Bacterial suspensions equivalent to a standard turbidity of 0.5 McFarland (1 to 2×10^8 CFU/ml) were prepared in sterile 0.9% saline (NaCl, w/v) from 18-hour-old pre-cultures (Muller Hinton broth, HIMEDIA, India) at 37˚C. Each suspension (2 mL) was used to flood a Muller Hinton agar plate (Himedia, India) poured into a Petri dish, as recommended by the French Society for Microbiology (SFM, 2024). After drying for a few minutes at room temperature, sterile 6 mm diameter Whatman No. 1 paper discs (Whatman International Ltd., England) were placed aseptically, using forceps, onto the surface of the previously flooded plate. The discs were then gently flooded with 20 µL of extract at a concentration of 20 mg/mL in Sterile Distilled Water (SDW). For each extract and each microbial strain, the experiment was duplicated, and a negative control was performed with SDW. The plates were then left at room temperature for 15 - 30 min before being incubated at 37˚C for 24 h. The diameters of the inhibition zones around the discs were measured in millimeters (mm) using a ruler. The degree of strain sensitivity to the extracts was estimated based on **Table 1**.

Table 1. Scale of sensitivity of microorganisms to extracts [14].

Diameter of the inhibition halo (Δ)	Degree of sensitivity of the germ
$\Delta < 7$ mm	Insensitive
$7 \text{ mm} \leq \Delta < 8$ mm	Sensitive
$8 \text{ mm} \leq \Delta < 9$ mm	Quite sensitive
$\Delta \geq 9$ mm	Very sensitive

2.5.3. Determination of Minimum Inhibitory Concentrations

The Minimum Inhibitory Concentrations (MICs) of the extracts on susceptible,

moderately susceptible, and highly susceptible strains were determined by the liquid microdilution method [15]. This method uses sterile 96-well microplates and iodo-dinitro-tetrazolium (INT, Sigma Aldrich, UK) as a cell viability indicator. For each assay, 50 µL of Mueller-Hinton broth was dispensed into each well. Then, 50 µL of the stock solution of the extract (320 mg/mL) was introduced into the first well of each column. A series of successive 1/2 dilutions was performed by transferring 50 µL from each well to the next, in order to obtain final concentrations ranging from 160 mg/mL to 0.078 mg/mL. Then, 25 µL of a standardized microbial suspension was added to each well. The microplates were incubated at 37˚C for 24 hours. After incubation, 50 µL of INT solution (0.2 mg/mL) was added to each well to reveal microbial growth. The plate was then incubated at 37˚C for 30 minutes. The MIC was defined as the lowest concentration of extract that did not produce any color change in INT (red/pink), indicating an absence of visible microbial growth. The experiment was duplicated for each extract and each microbial strain.

2.5.4. Determination of Minimum Bactericidal Concentrations

Minimum Bactericidal Concentrations (MBCs) were determined by inoculating the contents of all wells after determining the MIC on Mueller Hinton agar (Himedia, India) poured into Petri dishes [15]. After 18 to 24 hours of incubation at 37˚C, the MBCs were read. The lowest concentration of the extract that did not allow any microorganism to survive corresponded to the MBC.

2.5.5. Determination of the Antimicrobial Profile of Extracts

The mode of action of the extract was determined by the MBC/MIC ratio [16]. An extract is considered bactericidal when the MBC/MIC ratio is less than or equal to four ($r \leq 4$). Conversely, it is considered bacteriostatic when this ratio is greater than four ($r > 4$).

The bacteriostatic effect results in a halt to bacterial multiplication without necessarily destroying the organism. It involves a sometimes reversible inhibition of certain biological functions necessary for the organism's metabolism, growth, and multiplication, without compromising all vital functions [17]. In contrast, the bactericidal effect results in the definitive destruction of the microorganism over a more or less long period. This destruction is linked to irreversible damage that prevents any further proliferation [18].

2.6. Statistical Analysis

The data (inhibition diameter, MIC, and MBC) were recorded in Microsoft Excel 2021. They underwent descriptive statistical processing (proportion, mean, and standard error). The graphs were created using GraphPad Prism version 10.

3. Results

3.1. Phytochemical Screening

Table 2 shows the presence (+) or absence (−) of different groups of secondary

metabolites. The presence of compounds such as tannins (catechins), flavonoids, anthocyanins, reducing compounds, and mucilage indicates that *Pouteria campechiana* has a phytochemical profile rich in antioxidants and antimicrobials (tannins, flavonoids, anthocyanins), as well as molecules capable of acting on inflammation, oxidative stress, wound healing, and emollient properties (mucilage).

Table 2. Results of qualitative phytochemical screening.

Chemical Compounds	*Pouteria campechiana* (01)
Tannins	+
Catechial Tannins	+
Flavonoids	+ Flavones
Anthocyanins	+
Leucoanthocyanins	−
Reducing Compounds	+
Saponins	−
Mucilages	+
Steroids	−
Terpenoids	−
Cardenolides	−
Coumarins	−
Anthracenes	−
Alacoids	−

+: Present; −: Absent.

3.2. Quantification of Phenolic Compounds

Total Polyphenol Assay

Table 3 below shows the total polyphenol, flavonoid, and condensed tannin contents expressed in standard microgram equivalents per mg of extract.

The standard used for tannins is gallic acid, with the equation for the calibration curve $y = 0.0972x + 0.0046$ and a regression R^2 of 0.995.

The standard used for tannins is pyrogallol, with the equation for the calibration curve $y = 0.0727x + 0.0283$ and a regression R^2 of 0.98.

The standard used for flavonoids is rutin, with the equation for the calibration curve... with a regression $R^2 = 0.999$.

The ethanolic extract is much richer in phenolic compounds than the aqueous extract: twice as many polyphenols, 1.5 times more flavonoids, and twice as many tannins. Ethanol extracts the antioxidant and anti-inflammatory molecules contained in *P. campechiana* more effectively. The compounds present are mostly nonpolar or intermediate, as they migrate more readily in ethanol. The ethanolic extract was more effective against *P. campechiana* than water.

Table 3. Polyphenol, flavonoid, and condensed tannin content of *Pouteria campechiana* leaves.

Content / Types of extracts	Total Polyphenol Content (µg Eq AG/mg of extract)	Flavonoid content (µg EqRUT/mg of extract)	Tannin Content (µg EqPYR/mg of extract)
Aqueous extract	9.597 ± 0.060	3.532 ± 0.007	5.906 ± 0.004
Ethonic extract	18.725 ± 0.364	5.586 ± 0.144	11.712 ± 0.082

3.3. Antioxidant Activity

Table 4 summarizes the IC_{50} values of our extracts and ascorbic acid, which are detailed in each of the three figures. The IC_{50} is the concentration required to inhibit 50% of free radicals.

The lower the IC_{50}, the more potent the antioxidant. The ethanolic extract has strong antioxidant activity (IC_{50} = 57 µg/mL). The aqueous extract is much less active (IC_{50} = 326 µg/mL). No extract is as potent as ascorbic acid, which is expected since it is a pure antioxidant.

Table 4. Summary of the IC_{50} values of ascorbic acid, aqueous extract, and ethanolic extract.

Extract	Correlation coefficient	Equation of the line	Lethal Concentration 50 IC_{50} (µg/mL)
Aqueous extract	0.9946	Y = −0.001X + 1.1953	326.3 ± 1.414
Ethanolic extract	0.9956	Y = −0.0066X + 1.3068	56.902 ± 0.161
Ascorbic acid	0.9988	Y = −0.1619X + 0.5856	0.023 ± 0.002

3.4. Susceptibility Profile of Strains to Extracts

Table 5 and Figure 1 and Figure 2 present the activity of aqueous and ethanolic extracts of *P. campechiana* leaves against reference strains, clinical strains, and food strains, as well as the degree of sensitivity of each type of extract to the microorganisms under study.

Aqueous and ethanolic extracts of *P. campechiana* leaves were evaluated against different categories of strains: reference strains (ATCC), food strains (ECA), and clinical strains (ECC). Overall, the results show that the aqueous extract is generally more active than the ethanolic extract, reflecting the presence of predominantly water-soluble antimicrobial compounds.

Table 5. Mean diameters of inhibition zones (mm) induced by aqueous and ethanolic extracts of *P. campechiana* leaves.

Micro-organisms		Inhibition diameters (mm)	
Origin	Strains	Aqueous	Ethanolic
Reference	*S. aureus* ATCC 29213	14.0 ± 1.02	14.0 ± 0.46
	P. aeruginosa ATCC 27853	12.5 ± 1.02	10.0 ± 0.46
	E. coli ATCC 25922	13.0 ± 1.02	8.5 ± 0.46
	C. albicans MHMR	11.5 ± 1.02	-
	L. monocytogenes ATCC 19114	14.5 ± 1.02	10.0 ± 0.46

Continued

Food	ECA1	-	-
	ECA2	14.5 ± 1.02	-
	ECA3	10.5 ± 1.02	-
	ECA4	-	-
	ECA5	12.5 ± 1.02	-
Clinical	ECC1	10.0 ± 1.02	10.0 ± 0.46
	ECC2	-	11.0 ± 0.46
	ECC3	9.5 ± 1.02	9.5 ± 0.46
	ECC4	9.0 ± 1.02	8.5 ± 0.46
	ECC5	-	-

ECA: Foodborne *E. coli*; **ECC:** Clinically acquired *E. coli*; **-:** No activity.

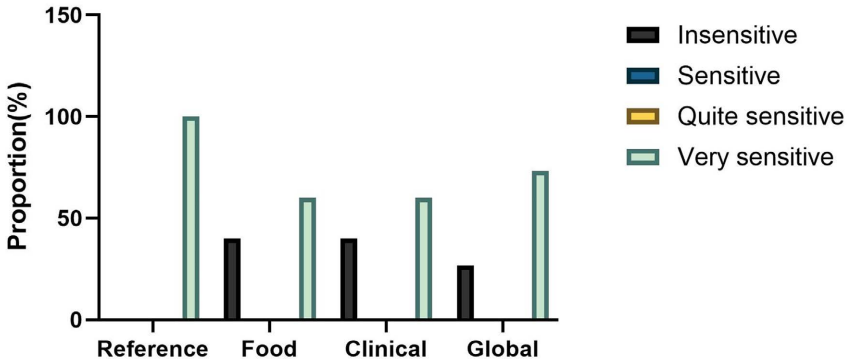

Figure 1. Degree of sensitivity of microorganisms to the aqueous extract of *P. campechiana*.

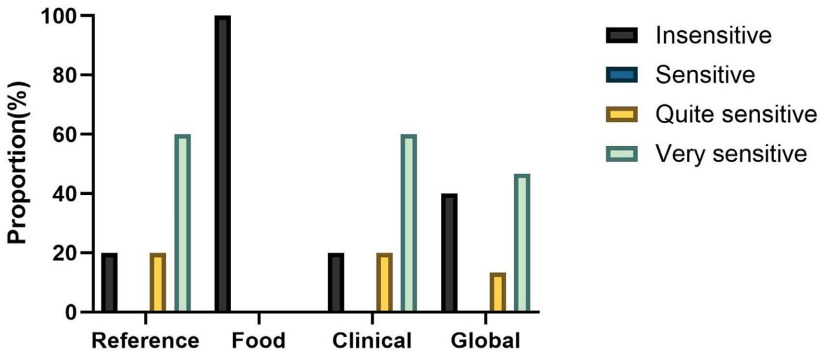

Figure 2. Degree of sensitivity of microorganisms to the ethanolic extract of *P. campechiana* leaves.

3.5. Antimicrobial Profile of the Extracts

The following **Table 6** illustrates the results of the minimum inhibitory and bactericidal concentrations of our extracts.

For the aqueous extract (**Table 6**), Minimum Inhibitory Concentration (MIC) values ranged from 20 to 40 mg/mL against all tested microorganisms. The lowest MIC value (20 mg/mL) was observed against *Candida albicans*, *Escherichia coli*

ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and several foodborne and clinical *E. coli* strains. No minimum bactericidal concentration (MBC) was detected at the highest tested concentration (160 mg/mL), with MBC values greater than 160 mg/mL for all microorganisms. Consequently, the MBC/MIC ratios were ≥ 4, indicating a bacteriostatic effect.

Table 6. MIC and MBC of the aqueous extract of *P. campechiana* leaves against microorganisms under study.

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Type of effect
Reference strain				
S. aureus ATCC 29213	40	>160	>4	Bacteriostatic
L. monocytogenes ATCC 19114	40	>160	>4	Bacteriostatic
E. coli ATCC 25922	20	>160	>4	Bacteriostatic
P. aeruginosa ATCC 27853	20	>160	>4	Bacteriostatic
Foodborne strains				
ECA2	40	>160	>4	Bacteriostatic
ECA3	20	>160	>4	Bacteriostatic
ECA5	40	>160	>4	Bacteriostatic
Clinical strains				
ECC1	20	>160	>4	Bacteriostatic
ECC3	20	>160	>4	Bacteriostatic
ECC4	20	>160	>4	Bacteriostatic

ECA: **Foodborne *E. coli*,** ECC: **Clinically acquired *E. coli*.**

Table 7. MIC and MBC of the ethanolic extract of *P. campechiana* leaves against the microorganisms under study.

Microorganisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Type of effect
E. coli ATCC 25922	40	>160	>4	Bacteriostatic
P. aeruginosa ATCC 27853	20	>160	>4	Bacteriostatic
S. aureus ATCC 29213	40	>160	>4	Bacteriostatic
L. monocytogenes ATCC 19114	40	>160	>4	Bacteriostatic
Clinical strains				
ECC1	20	>160	>4	Bacteriostatic
ECC2	10	>160	>4	Bacteriostatic
ECC3	10	>160	>4	Bacteriostatic
ECC4	10	>160	>4	Bacteriostatic

ECA: Foodborne *E. coli*, **ECC:** Clinically acquired *E. coli*.

For the ethanolic extract (Table 7), MIC values ranged from 10 to 40 mg/mL. The lowest MIC values (10 mg/mL) were recorded for the clinical *E. coli* strains

ECC2, ECC3, and ECC4. As observed with the aqueous extract, MBC values exceeded 160 mg/mL for all tested strains, resulting in MBC/MIC ratios greater than 4. All tested microorganisms exhibited a bacteriostatic response to the ethanolic extract. **Table 6**: MIC and MBC of the aqueous extract of *P. campechiana* leaves against microorganisms under study.

4. Discussion

This study aimed to evaluate the biological potential of *Pouteria campechiana* leaves through their phytochemical composition, antioxidant activity, and antimicrobial properties. The results obtained generally confirm data from the literature, while providing new insights into the comparative performance of aqueous and ethanolic extracts.

Phytochemical screening revealed an abundance of tannins, flavonoids, anthocyanins, reducing compounds, and mucilage. These results are consistent with those of previous studies reporting that *Sapotaceae* species, particularly *P. campechiana*, are rich in polyphenols and natural pigments responsible for their biological properties [19]. Quantification shows that the ethanolic extract contains approximately twice as many polyphenols (9.6 vs. 18.7 µg AG eq/mg), 1.5 times more flavonoids (3.53 vs. 5.58 µg RUT eq/mg), and twice as many tannins (5.9 vs. 11.7 µg PYR eq/mg) as the aqueous extract. This result is consistent with the fact that ethanol preferentially extracts semipolar metabolites, particularly flavonoids, condensed tannins, and reducing phenolic compounds [20]. These data also corroborate the work of [21], who reported a high concentration of polyphenols in the leaves and fruits of *P. campechiana*, considered one of the richest species in carotenoids and flavonoids within the *Sapotaceae* family.

Antioxidant evaluation by DPPH revealed a clear superiority of the ethanolic extract (IC_{50} = 56.9 µg/mL) compared to the aqueous extract (IC_{50} = 326.3 µg/mL). This trend is strongly correlated with the high polyphenol and tannin content of the ethanolic extract, confirming the major role of these compounds in neutralizing free radicals [5]. Although less potent than ascorbic acid (IC_{50} = 0.023 µg/mL), which is expected given that it is a pure standard, the ethanolic extract demonstrates notable activity comparable to that reported in other tropical medicinal plants rich in antioxidant metabolites [22]. These results suggest the potential use of the ethanolic extract of *P. campechiana* as a natural source of antioxidants, which could help reduce oxidative stress, known to be involved in various chronic diseases [5].

Both aqueous and ethanolic extracts showed moderate activity against all the microbial strains tested. The aqueous extract was the most active in agar diffusion, with zones of inhibition ranging from 9 to 14.5 mm depending on the strain, while the ethanolic extract showed weaker activity, limited to a few reference and clinical strains. This observation can be explained by the better diffusion of water-soluble compounds in the agar [13], as well as by the presence of tannins and mucilage capable of precipitating bacterial membrane proteins [23].

Susceptible strains include Staphylococcus aureus, Listeria monocytogenes, E. coli ATCC 25922, and certain foodborne strains (ECA2, ECA3, ECA5). Clinical strains, which are highly multidrug-resistant, show lower susceptibility, consistent with the literature indicating that hospital strains often express enhanced resistance mechanisms [5].

MIC values are generally high (10 - 40 mg/mL), and MBC values are greater than 160 mg/mL for all strains. The MBC/MIC ratio is consistently > 4, indicating a purely bacteriostatic effect for both extracts.

This type of effect is common for plant extracts rich in tannins and flavonoids, whose action is based primarily on the inhibition of microbial growth, the disruption of membrane permeability, the chelation of ions essential for growth, and the inhibition of metabolic enzymes [24]. However, the lower MICs observed for some clinical strains (e.g., ECC2 - ECC4, MIC = 10 mg/mL) suggest the presence of compounds with targeted activity, paving the way for further fractionation of the extracts. Thus, the ethanolic extract appears more promising for antioxidant applications, while the aqueous extract could be better utilized for local antimicrobial applications (solutions, infusions, topical formulations).

The observed antioxidant and antimicrobial properties confirm the traditional uses of *P. campechiana* in the treatment of infections and inflammation [25] [26]. However, the high MICs and the predominance of a bacteriostatic effect indicate that direct therapeutic use in its crude form remains limited.

Future studies could include the isolation of bioactive molecules (LC-MS/MS, GC-MS, HPLC), synergistic studies with antibiotics, particularly against ESBLs, in vivo testing for pharmacological validation, cytotoxicity assessment for safety, and the formulation of bioproducts (standardized extracts, gels, syrups, food additives).

5. Conclusions

This study aimed to evaluate the biological potential of *Pouteria campechiana* leaves through their phytochemical composition, antioxidant activity, and antimicrobial properties. The results revealed a significant presence of secondary metabolites such as tannins, flavonoids, anthocyanins, reducing compounds, and mucilage, confirming the plant's rich biochemical profile and supporting some of its traditional uses.

Quantification of secondary metabolites shows that the ethanolic extract is richer in polyphenols, flavonoids, and tannins than the aqueous extract, indicating better extraction of semipolar compounds by ethanol. This difference is also reflected in antioxidant activity: the ethanolic extract exhibits superior free radical scavenging capacity, although it remains lower than that of ascorbic acid, which was used as a reference.

From an antimicrobial standpoint, the tested extracts primarily exhibited a bacteriostatic effect on the microorganisms studied. The aqueous extract was distinguished by better diffusion on agar plates, generating larger zones of inhibition

against certain strains, particularly *Staphylococcus aureus*, *Listeria monocytogenes*, and some foodborne strains. Nevertheless, activity remained moderate, with relatively high MICs and reduced efficacy against multidrug-resistant clinical strains.

In summary, the leaves of *P. campechiana* show significant interest as a source of bioactive compounds with antioxidant properties and a moderate antimicrobial effect. These results suggest potential uses in phytotherapeutic or agri-food applications, particularly as complementary natural agents. However, further studies, including the isolation of active molecules, the evaluation of mechanisms of action, and *in vivo* testing, are necessary to confirm and optimize the intended applications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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