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Agbangnan Dossa Cokou Pascal

Laboratory of Studies and Research in Applied Chemistry, Ecole Polytechnique d'Abomey, University of Abomey, Calavi, Benin

Daye Efloric Raphaël

Laboratory of Studies and Research in Applied Chemistry, Ecole Polytechnique d'Abomey, University of Abomey, Calavi, Benin

Koudoro Yaya Alain

Laboratory of Studies and Research in Applied Chemistry, Ecole Polytechnique d'Abomey, University of Abomey, Calavi, Benin

Olayé Théophile

Laboratory of Studies and Research in Applied Chemistry, Ecole Polytechnique d'Abomey, University of Abomey, Calavi, Benin

Correspondence:

Dr. Agbangnan Dossa Cokou Pascal
Laboratory of Studies and Research in Applied Chemistry, Ecole Polytechnique d'Abomey, University of Abomey, Calavi, Benin
Email: cokou2010@gmail.com

Secondary metabolites, antiradical and antibacterial activities of *Pteleopsis suberosa* leaves and trunk bark used in Benin to treat toothache

Agbangnan Dossa Cokou Pascal, Daye Efloric Raphaël, Koudoro Yaya Alain, Olayé Théophile

ABSTRACT

Among the most common health problems worldwide, toothache is often cited and is often treated with herbal medicines to relieve the pain and inflammation associated with it. The present work reports the preliminary phytochemical analysis, antiradical and antibacterial activities of leaves and trunk bark of *Pteleopsis suberosa*, a plant highly sought after in traditional medicine in Benin to treat toothache. Secondary metabolites were identified by staining and precipitation reactions specific to each metabolite family. Total phenols were determined by the Folin Ciocalteu method. The aluminum trichloride method was used to quantify total flavonoids, while the determination of condensed tannins was carried out by the hydrochloric vanillin method. The antiradical activity of the extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl and the antibacterial activity by the dilution method in microplates and Petri dishes. Leaves and bark of *Pteleopsis suberosa* trunk contain tannins, flavonoids, anthocyanins, leuco-anthocyanins, a reducing compound. Ethanol extract of *Pteleopsis suberosa* leaves ($IC_{50} = 0.0015 \mu\text{g}/\mu\text{L}$) showed more interesting anti-radical activity than butylhydroxytoluene ($IC_{50} = 0.012 \mu\text{g}/\mu\text{L}$), butylated hydroxyanisole ($IC_{50} = 0.016 \mu\text{g}/\mu\text{L}$) and Trolox ($IC_{50} = 0.013 \mu\text{g}/\mu\text{L}$). The ethanolic extract of the bark of the trunk of *Pteleopsis suberosa* showed bactericidal activity against the *Staphylococcus aureus* strain.

Keywords: *Pteleopsis suberosa*, Metabolite, Antiradical, Bactericidal, Toothache.

INTRODUCTION

The most common chronic diseases worldwide are dental caries and periodontal disease [2]. The World Health Organization has identified oral and other systemic diseases as serious non-communicable diseases. [2] The body's most vital organs are the teeth, and tooth decay contributes significantly to dental problems. The process of tooth decay includes 4 stages, starting with a small black spot in stage 1, followed by enamel breakdown 2, nerve damage in stage 3, and destruction of the pulp with pus. Repair of dental caries can be achieved through simple treatment steps 1 and 2, but if the initial steps are not assessed or ignored, tooth decay can progress to steps 3 and 4 [3]. Toothache, inflammation and acute osteomyelitis are among the resulting complications. Preventive measures are necessary to effectively manage tooth decay. Early diagnosis and early management of dental caries are essential to good dental health. Good dental health requires early diagnosis and prompt treatment of dental caries. The occurrence of dental caries is due to the action of Gram-positive acidogenic and aciduric bacteria, such as Streptococcus, Lactobacillus and Actinomycetes species, which convert sucrose into organic acids that dissolve calcium phosphate in teeth [4]. Dental practitioners have used medicinal plants for a long time, and they have had this use in practice throughout history [5]. Traditional plants and natural products have been used in many cases to treat toothache. In Benin, people used various medicinal plants to relieve toothache. Herbs commonly used to treat dental diseases include, among others, *Pteleopsis suberosa*. *Pteleopsis suberosa*, a shrub belonging to the *Combretaceae* family, is characterized by its characteristic bark and corky warts [6]. It can reach heights of up to 10 m. In West Africa, from Senegal to Benin, it is found without restriction. West Africa used the various components of *Pteleopsis suberosa* for its medicinal properties. The usual use of fresh roots as a remedy, antiseptic treatment and medicinal cream is to treat dermatitis [7]. Extracts of roots and young shoots are considered cough suppressants. The use of the bark or leafy twig of *Pteleopsis suberosa* to treat skin infections, fever, filariasis, sores and conjunctivitis is also popular as an ethnomedical treatment. [6-8]. Triterpenoids, coumarins and tannins are considered important phytonutrients in this region [9]. In order to optimize the use of *Pteleopsis suberosa* in traditional medicine, this study aims to characterize the bioactive compounds, the anti-radical and antibacterial activities of extracts from these organs.

MATERIALS AND METHODS

MATERIALS

The leaves and bark of the trunk of *Pteleopsis suberosa* from southern Benin constitute the plant material. The reference strain *Staphylococcus aureus* ATCC 6538 and the strain clinical *Staphylococcus epidermidis* constitute the bacterial material.

METHODS

After harvest, the leaves and trunk bark of *Pteleopsis suberosa* were subjected to pretreatment and dried at room temperature until their mass was stabilized, followed by powdering. The preparation of the ethanolic extract of *Pteleopsis suberosa* was produced under ultrasound by mixing 10 grams of powdered biomass with 100 mL of solvent. The mixture was then sonicated using the Bandelin (Sonorex device Digitech) for two hours at 50°C. Additionally, the extracts were filtered using Whatman No. 1 filter paper and then subjected to vacuum concentration at 50 °C ± 1 °C.

Quantitative determination of phenolic compounds

Content of total phenolic compounds: Colorimetric measurement with Folin Ciocalteu was used with some adjustments to determine the actual amount of phenolic compound. In this technique, a mixture of the two acids (phosphotungstic and phosphomolybdic) was used to reduce phenols, which in turn were oxidized to create tin blue oxide with molybdenum. Finally, the absorbance was measured at 760 nm using a suitable spectrophotometer and the total total phenol content is expressed in micrograms of gallic acid equivalent per mL (gGAE /mL) [10, 14].

Total content in flavonoids: Using aluminum trichloride (AlCl₃), the total flavonoid content was determined by complexation to yield complexes of flavonoids and aluminum. A spectrophotometer was used to measure the absorbance at 415 nm and from which the total flavonoid content expressed in micrograms was determined. equivalent of quercetin per mL of extract (μ gQE /mL) [15, 16].

Content in condensed tannins: To determine the amount of condensed tannin, the vanillin hydrochloric acid method was used. At 500 nm, the absorbance was measured by a spectrophotometer and the catechin equivalent content (μg EC/ mL) was considered as condensed tannin content.

DPPH free radical scavenging assay: The 96-well microplates were used for the determination of antiradical activity. Thus, in the five wells of the first two lines of the microplate reader were deposited 100 μL of ethanolic extract of leaves and of trunk bark of *Pteleopsis suberosa* then follows a two-fold dilution with methanol, from the second to the last line (8th line), where 100 μL of dilution was released in each well. Then 100 μL of methanolic solution of DPPH prepared at a concentration of 0.1 mg/ mL were added to the first three wells of each row while 100 μL of methanol was added to the two well remaining to serve as a negative control. As for the positive control, it was made by mixing 100 μL of methanol with 100 μL of DPPH solution. Finally, the plates were incubated away from light for one hour and the absorbance was read 15 minutes later [10, 14].

$$P (\%) = \frac{[A_{\text{posi contr}} - (A_{\text{sam}} - A_{\text{nega contr}})]}{A_{\text{posi contr}}} \times 100$$

Antibacterial activity of the extract from leaves and stems bark of *Pteleopsis suberosa*

Bacterial sensitivity test of the two extracts of *Pteleopsis suberosa*:

The sensitivity tests of bacterial strains to the extract ethanolic of *Pteleopsis suberosa* implied the addition from 100 μL of bacterial inoculum to 100 μL of concentrated extract at 20 mg/ mL. Then, the microplate was incubated for 24 hours at 37°C and then 40 μL of iodinitrotetrazolium (INT) at 0.2 mg / mL were added to each concentrated solution followed a further incubation of 30 min. The extract is considered inactive if a pink or red color appears in the well. Three tests were carried out for each concentration [17].

Determination of the minimum inhibitory concentration (MIC):

The MICs were determined by testing on the two strains (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis*) a concentration range of the two plant extracts between 10 mg/ mL and 0.15 mg/ mL in a 96-well microplate. To ensure the reliability of the results, the tests were repeated three times. The method consists of initially adding 100 μL of Mueller Hinton broth to each well. Then we add 100 μL of the most concentrated solution of extract at 20 mg/ mL in the first two wells. From the second well, successive two-fold dilutions were carried out until the last well. Finally, 100 μL of bacterial broth at 10.6 CFU/ mL is added to the contents of each well. We then carry out an incubation of twenty (20) hours at 37°C. After incubation, 40 μL of iodinitrotetrazolium at 0.2 mg/ mL was added to each well. The presence of stains in a well proves that there has been bacterial growth. The MIC corresponds to the lowest concentration from which there is no color change in the well after the addition of iodinitrotetrazolium [17].

Determination of the minimum bactericidal concentration (MBC):

The MBC was determined by inoculating 10 μL of wells in which no bacterial growth was observed on of agar Mueller Hinton. After 24 hours of incubation, the lowest concentration of extract from which was absolutely no bacterial growth on medium agar corresponds to the MBC [17].

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Metabolites identified in the organs (leaves and trunk bark) of *Pteleopsis suberosa* are listed in Table 2. The leaves and trunk bark of *Pteleopsis suberosa* contain tannins, flavonoids, anthocyanins, leucoanthocyanins, reducing compounds and anthraquinones while mucilages and saponosides have only been identified in the trunk bark. On the other hand, sterols and terpenes were found in the leaves. Our results are in agreement with the work of Lagnika *et al* [9] at the level of *Pteleopsis suberosa* leaves harvested in Benin. On the other hand, in Mali, Sanogo [18] identified coumarins, saponosides and mucilages absent in the Benin sample. The variation in secondary metabolites observed in our samples compared to studies previous may be linked to the harvest period, soil properties or climatic factors [19]. The presence of secondary metabolites such as flavonoids, anthocyanins, terpenes and mucilages could justify the use of this plant in traditional medicine to treat toothache as reported in the literature [20].

Table 1: Methods for identifying secondary metabolites from leaves and trunk bark of *Pteleopsis suberosa*

Secondary metabolites	Chemical test
Alkaloids	Mayer test and Dragendorff test
Anthocyanins	test with hydrochloric acid and ammonia
Anthraquinones	Bornstranger test
Coumarins	Fluorescence at 365 nm
Flavanoids	shinoda test and magnesium powder
Tannins	stiasny test,
Saponins	Foam test
Leuco anthocyanins	Bate -Smith and Metcalf
Mucilages	puff test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling test
Sterols and terpenes	Liebermann- Burchard test
proteins	Biuret test

Table 2: Secondary metabolites identified in *Pteleopsis suberosa* leaves and the bark of the trunk

Secondary metabolites		<i>Pteleopsis suberosa</i>	
		Leaves	Trunk bark
		Observation	
Tannins	catechists	present	present
	Gallic	present	present
Flavanoids		present	present
Anthocyanins		present	present
Leuco anthocyanins		present	present
Reducing compound		present	present
Mucilages		absent	present
Coumarins		absent	absent
Alkaloids		absent	absent
Cyanogenic derivatives		absent	absent
Saponosides		absent	present
Anthraquinones		present	present
Sterols and terpenes		present	absent

Phenolic compound content: The contents of phenolic compounds in the ethanolic extract of leaves and trunk bark of *Pteleopsis suberosa* are recorded in Table 3. The highest total phenolic content is obtained in the ethanolic extract of *Pteleopsis suberosa* trunk bark (203.88 ± 6.31) $\mu\text{GAE} / \text{mgDM}$. The leaves of this plant have a total phenol content of $190.37 \pm 6.12 \mu\text{GAE} / \text{mgDM}$. On the other hand, regarding the content of total flavonoids, the highest content is recorded at the leaf level (5.76 ± 0.17) $\mu\text{QE} / \text{mgDM}$ compared to a content of $2.43 \pm 0.24 \mu\text{QE} / \text{mgDM}$ for the bark of the trunk. The tannin content of *Pteleopsis suberosa* leaves is 7.519 ± 1.304 $\mu\text{CE} / \text{mgDM}$ while in the bark of the trunk there is a content of $11,929 \pm 1,296 \mu\text{CE} / \text{mgDM}$.

Table 3: Content of phenolic compounds in the ethanolic extract of *Pteleopsis suberosa* leaf and trunk bark

Ethanol extract	Total phenol content ($\mu\text{GAE} / \text{mgDM}$)	Total flavonoid content ($\mu\text{QE} / \text{mgDM}$)	Condensed tannin content ($\mu\text{CE} / \text{mgDM}$)
L Ps	190.37 ± 6.12	5.76 ± 0.17	7.519 ± 1.304
T Ps	203.88 ± 6.31	2.43 ± 0.24	11.929 ± 1.296

Legend: $\mu\text{GAE} / \text{mgDM}$: microgram equivalent of gallic acid per gram of dry matter; $\mu\text{QE} / \text{mgDM}$: microgram Quercetin Equivalent per milligram of dry matter; $\mu\text{CE} / \text{mgDM}$: microgram catechin Equivalent per milligram of dry matter; **LP.s**: *Pteleopsis suberosa* leaves; **TP.s**: bark of the trunk of *Pteleopsis suberosa*

DPPH Free Radical Removal Test: Extent of elimination of the radical free DPPH after 15 minutes, 30 minutes, 45 minutes and 60 minutes of reaction is a function of their concentration of ethanolic extract of leaves and stem bark of *Pteleopsis suberosa* and the results are presented in Figure 1. After 15 minutes of reaction, we see that the reaction is complete. For all concentrations of this extract tested, it has had a gradual increase in the rate of radical scavenging before THE trays born be observed. These curves were used to determine the concentration of the extract resulting in inhibition of 50 % of DPPH radicals. More this concentration (IC_{50}) is weak, more the activity anti-radical East interesting. Ethanol extract of *Pteleopsis suberosa* leaves showed more interesting anti-radical activity than butylhydroxytoluene ($\text{IC}_{50} = 0.012 \mu\text{g} / \mu\text{L}$), butylated hydroxyanisole ($\text{IC}_{50} = 0.016 \mu\text{g} / \mu\text{L}$) and Trolox ($\text{IC}_{50} = 0.013 \mu\text{g} / \mu\text{L}$) which are synthetic antioxidants. This interesting anti-radical activity of *Pteleopsis suberosa* is linked to the content of phenolic compounds in this plant [21]. Aladodo *et al* [7] showed at the level of the aqueous extract of the trunk of *Pteleopsis suberosa* bark from Nigeria has an interesting anti-radical activity ($\text{IC}_{50} = 0.002 \mu\text{g} / \mu\text{L}$). The most plausible mechanism of action of phytochemicals for the treatment of toothache would be their antioxidant activity [22, 23].

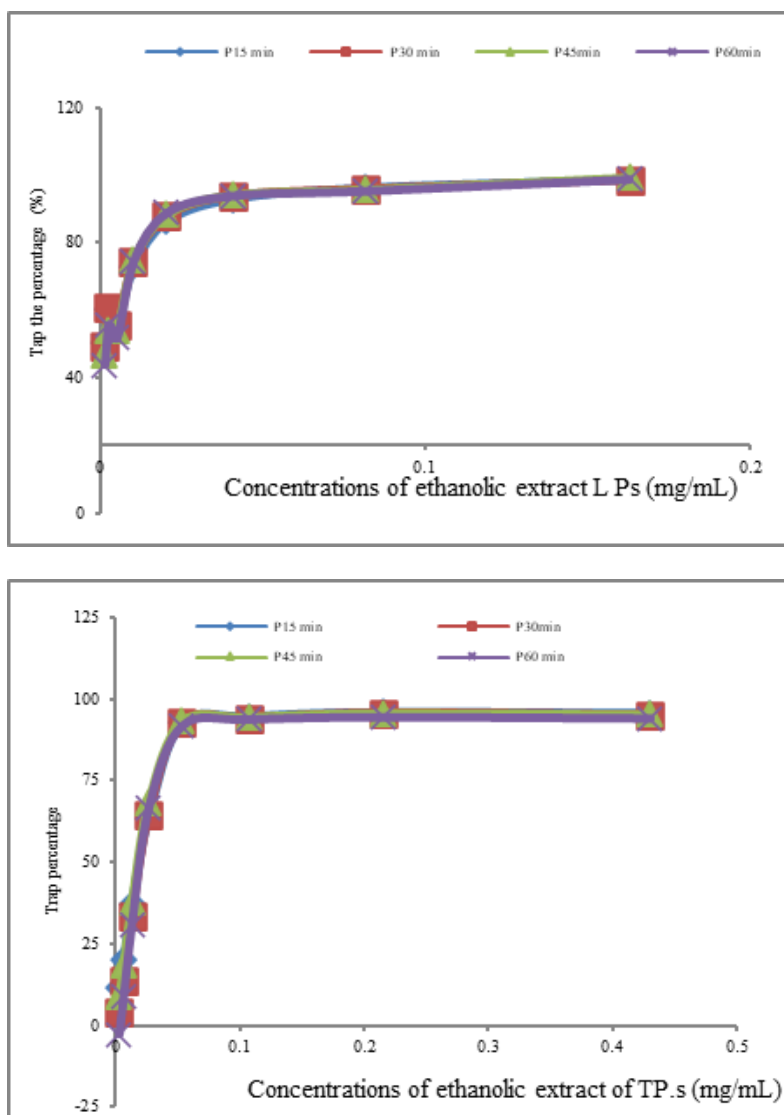


Figure 1: Antiradical activity of ethanolic extract of *Pteleopsis suberosa* organs

Antibacterial activity of *Pteleopsis suberosa* leaf and Trunk bark extracts:

The Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentrations (MBC) of extracts (ethanolic and hydroethanolic) of the leaves and bark of the trunk of *Pteleopsis suberosa* are recorded in Table 4. The ethanolic and hydroethanolic extracts of *Pteleopsis suberosa* leaves inhibited *Staphylococcus aureus* and *Staphylococcus epidermidis* strains at a concentration of 5 mg/ mL. The minimum inhibitory concentration of ethanolic and hydroethanolic extracts of the trunk bark of *Pteleopsis suberosa* against the strain of *Staphylococcus aureus* is 5mg/ml while with the strain of *Staphylococcus epidermidis*, the concentrations of the ethanolic and hydroethanolic extracts are 5 mg/ mL and 10 mg/mL,

respectively. Only the ethanolic extract of the trunk bark of this plant showed bactericidal activity against the strain of *Staphylococcus aureus* at a concentration of 10 mg/ mL. Bactericidal activity of *Pteleopsis suberosa organ* extracts observed against the strain of *Staphylococcus aureus*, could be explained by the richness of this plant in secondary metabolites, in particular in flavonoids, tannins, anthocyanins, saponins and phenols either individually or by synergistic effect between them [24, 25]. The interesting antimicrobial activity obtained here could be due to the presence of flavonoids and tannins in the extracts [26].

Table 4: MIC and MBC of *Pteleopsis suberosa* leaf and trunk bark extracts

Strains	Excerpts	<i>Pteleopsis suberosa</i>			
		Leaves		Trunk bark	
		MIC (mg/ mL)	MSC (mg/ mL)	MIC (mg/ mL)	MBC (mg/mL)
<i>S. aureus</i>	Ethanol	5	10>	5	ten
	Hydroethanolic	5	10>	5	10>
<i>S. epidermidis</i>	Ethanol	5	10>	5	10>
	Hydroethanolic	5	10>	ten	10>

Legend: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; *S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*

CONCLUSION

The medicinal value of the plant lies in the active chemical substances present in the plant. It is therefore important to guarantee the quality and standards of plant material. The present study aims to evaluate the phytochemical analysis, antiradical and antimicrobial activities of *Pteleopsis suberosa* leaf and stem extracts. It appears that the leaves and bark of *Pteleopsis suberosa* contain chemical metabolites that have shown antibacterial and antiradical activities that may justify their use for the treatment of dental infections. Indeed, the leaves and bark of the trunk of *Pteleopsis suberosa* contain tannins, flavonoids, anthocyanins, leucoanthocyanins, reducing compounds and anthraquinones while mucilages and saponosides have only been identified in the trunk bark. On the other hand, sterols and terpenes were found in the leaves. We can report that *Pteleopsis suberosa* could have antibiotic activity as it exhibited potent antibacterial and antioxidant activities. The organs of *Pteleopsis suberosa* constitute a potential source of active ingredients. These results provide a scientific basis for traditional uses of *Pteleopsis suberosa* in traditional medicine.

Conflict of Interest Disclosure

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

ORCID ID

Agbangnan Dossa Cokou Pascal: <https://orcid.org/0000-0002-9093-7731>

Daye Efloric Raphaël: <https://orcid.org/0009-0008-2401-6038>

Koudoro Yaya Alain: <https://orcid.org/0000-0001-9879-5523>

Olaye Théophile: <https://orcid.org/0009-0004-0177-3758>

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