Phytochemical Screening and Potential antibacterial, antifungi and antioxidative activities of the leaves extracts of *Piperomia pellucida* (Piperaceae)

ABSTRACT

The hexane, ethanolic, hydroethanolic and aqueous leaves extracts of *Piperomia pellucida* were evaluated *in vitro* for their antimicrobial activity against five gram negative bacilli (*Klebsiella pneumoniae* CHUY, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* CHUY and *Salmonella enterica* CHUY), two cocci gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 5199) and two fungi (*Candida albicans* CHUY and *Candida albicans* 20231) using microdilution techniques. The extracts were also assayed for their antioxidant properties by DPPH and FRAP methods with Vitamin C as reference. The results demonstrated that, the hexane extract exhibited strong antibacterial potency with MIC value of 0,625 mg/mL and MBC of 1,25 mg/mL on *Salmonella typhi* CHUY. Also, it showed good activities on *P. aeruginosa* ATCC 27853 and *S enterica* CHUY; and weak activity against *E. coli* ATCC 35218 and *S. aureus* ATCC 25923. The aqueous extract displayed good activities against *S. aureus ATCC 25923*, *P. aeruginosa ATCC* 27853, *E. coli ATCC* 35218, *S. typhi CHUY*, *S enterica CHUY* ; weak potency toward *E. coli* ATCC 35218 and was inactive on the tested fungi strains. The ethanolic and hydroethanolic extract displayed weak activities on *E. coli ATCC 35218*, *S enterica CHUY* and *S. typhi CHUY*.

The strongest antioxidative activities of effiecient concentrations were exhibited by the ethanolic (10,681 ± 0.530µg/mL) and the hydroethanolic extracts (16,339 ± 0.043µg/mL).

Keywords: Piperaceae, *Piperomia pellucida,* antibacterial, antifungi, antioxidative

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1-Introduction

Infectious diseases are those cause by bacterias, viruses, fungi and parasites, such as hepatitis, gastroenteritis, influenza, COVID-19 and common cold (Barreto and al., 2006). During the infectious process, free radicals are produced by phagocytic cells. These are naturally beneficial for the protection of the body against attacks of pathogens. However,

when produced in large quantities, it leads to oxidative stress responsible for inflammatory conditions, diabetes, atherosclerosis, high blood pressure, cardiovascular problems, Parkinson's, Alzheimer, cancer and other opportuninstic diseases (Haleng et *al.*, 2007). About 90% of the death cases from these diseases comes from Africa (Académie des sciences, 2006). For instance, in Cameroon in 2012, 32,000 of deaths cases was due to HIV infections, while respiratory infections accounted for 29,100 deaths (WHO, 2012). It worthy of note that, in 2017, the Global Antimicrobial Resistance Surveillance System (GLASS) reported that, the pénicillin resistance of *Streptococcus pneumoniae* varied from 0 to 80% and the Ciprofloxacin resistance of *Escherichia coli* from 8 to 65% (WHO, 2017; Ibe-diala and Igwe OU, 2022; Ho et al., 2022). Based on the resistances exerted by bacterias on these drugs, there is need to search for alternative sources of antibacteria agents. About 80% of the world's population use medicinal plants for basic healthcare needs (Mahtab, 2016). Interstingly, plant of the Piperaceae family, are amongst the different herbs used for the treatment of microbial infections. For intance, several species of this family have been reported for their therapeutic effects against asthma, microbial infections and respiratory disorders (Casalinuovo et *al.*, 2004).

Peperomia pellucida (Linn.), locally known as *Luchi Pata*, is an annual herb (Ghani et *al.*, 1998) widely distributed in tropicale zone of Central and South America, Asia and Africa (Mozambique, Angola and Cameroon) (Arrigoni-Blank et *al.*, 2004 ; Santos et *al.*, 2001). The plant is eaten as salad or as cooked vegetable. It is used to treat glaucoma, lower blood chlolesterol, abscesses, skin wounds and eye inflammation (Bojo, 1994). Also, it serves as remedy for insect bites, sexually transmitted diseases (Page, 1981), cough (Gasparini, 1995), small pox (Moreton, 1999 ; Ragasa et *al.*, 1998), measles (Smitinand, 1980), kidney infection (Bojo, 1994 ; Antoniolli et *al.*, 2004), analgesic (Antoniolli et *al.*, 2004), proteinuric, and diuretic (May, 1982), cataract and conjunctivitis (May, 1982 ; Khan and Omoloso, 2002). The leaves and stems decoction are used to treat gout, arthritis, heal wounds, acne, boils and pimples (Khan and Omoloso, 2002). The leaves alone is used as remedy for the treatment of fever, eczema, abdominal pains, and convulsions (Majumder et al., 2011 ; Aksozek and al., 2002) ; while the whole plant is applied for treating colic, fatigue, gout, headache, rheumatic and joint pain (Aksozek and al., 2002).

Previous chemical and pharmacological studies carried out on *P. pellucida* revealed the presence of alkaloids, cardenolides, saponins, tannins (Mishra, 2010), essential oils, flavonoids, phytosterols, phenylpropanoids and benzoic acids (Seeram et *al.*, 1998). Some of

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the constituents displayed antibacterial, antiinflammatory, analgesic (Aziba and *al*., 2001; Khan and Omoloso, 2002), antifungal, and anticancer activities (Ragasa and *al*., 1998 ; Xu and *al*., 2006). Thus, in continuation of our work on this plant, the present study seeks to evaluate the antimicrobial, antifungi and antioxidant activities of the hexane, ethanolic, hydro-ethanolic and acqueous extracts of *P. pellucida* against some gram negative and positive bacterial strains.

2. Materials and methods

2.1. Plant material

The whole plant of *P. pellucida* (Piperaceae) was collected at Mbocko, in the Littoral region of Cameroon. The species was authenticated at the National Herbarium of Cameroon by comparison with the botanical specimen C.N.A.D n° 1390 and the attribution of the identification number 23846/SRF.

2.2. Extraction

The leaves were air dried and crushed to afford 140g of powder. This was shared in four parts of 34g each. And macerated seprately at room temperature in hexane (1L), ethanol (1L), hydroethanolic mixtrues (7:3) (1L) and distilled water (1L) for 48 hours. The hexane and ethanolic portions were filtered and concentrated using a vacuum evaporator to yeild 1,28 and 2,4g of the extracts, respectively. The hydroethanolic and aqueous portions were lyophilised yielding 3 and 4g of the extracts, respectively.

2.3. Phytochemical screening of the crude extract

The extracts were screened for the presence of different classes of compounds using Libermann-Buchard test for sterols and terpenes, Meyer test for alkaloids, Sodiate test for coumarins, Foam Index test for saponins, $FeCl_3$ test for phenolic compounds as well as tanins and flavonoids. These phytochemical screening were carried out using standard methods, as described in the literature (Ibibia, 2012; Harbone, 1973; Harbone and Baxter, 1993; Ogboru et al., 2015) and all observations were recorded.

2.3.1. Characterisation of steroids and triterpens

1 mL of concentrated sulfuric acid is mixed with 2mL of extract in a test tube. The appearance of red or green colorations indicated the presence of triterpenoids ~~n~~or steroids (Ibibia, 2012).

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2.3.2. Characterisation of alkaloïds

In a test tube, 5 mL of the extract are disolve in 1 mL of methanol. 1mL of 1% sulfuric acid is added and heated for 5minutes. After cooling and fitration, 5 drops of Mayer reagent was introduced. The formation of a white precipitate confirmed the presence of alkaloids.

2.3.3. Characterisation of coumarins

2 mL of dilute sodium hydroxide was added to the extract in a test tube and heated in water bath for a few minutes. It was spoted on a thin layer chromatographic plate and observed with a UV lamp of 254 and 365 nm. A yellow-green coloration indicates the presence of coumarins in the extract (Ibibia, 2012).

2.3.4. Characterisation of saponins

2mL of distillated water was added to 2mL of the extract and shoked vigourously. The formation of a persistant foam confirms the presence of saponins (Ibibia, 2012).

2.3.5. Characterisation of tanins

2 mL of 5 % ferric chloride was added to 1mL of the extract in a test tube. The appearance of a green precipitate indicates the presence of tanins (Ibibia, 2012).

2.3.6. Characterisation of flavonoïds

1mL of sodium hydroxide was mixed with 3 mL of the extract. The appearance of yellow colouration indicate the presence of flavonoids (Ibibia, 2012).

2.4. Biologicals activities

2.4.1. Antimicrobial activity

The disk method was used to evaluate the antimicrobial potency and the microdilution method for the Minimal inhibitory concentration (MIC), Minimal bacteriocidal concentration (MBC) and the Minimal fongicidal concentration (MFC) as well as the ratios of MBC/MIC, MFC/MIC.

2.4.2. Preparation of stock solution

10mg/ml of the stock solution of the extracts were prepared and stored at 4°C for testing. 2mg/ml each of Ciprofloxacin and amphotericin B used as positive controls, were dissolved in sterilized distilled water under the same conditions. The final concentration of

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extracts and reference antimicrobial in the first line of the microdilution plate was calculated using following formular.

$$C(i) \times Vi = Cf \times Vf \Rightarrow Cf = (Ci \times Vi)/Vf$$

Ci: Initial concentration; Cf: Final concentration; Vi: Initial Volume; Vf: Final Volume

2.4.3. Microorganisms and reference antibiotics used

The antimicrobial activity was carried out on nine microbials strains among which, were five gram negative (*Klebsiella pneumoniae*, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* and *Salmonella enterica*); two gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 5199) and two fungi (*Candida albicans* CHUY and *Candida albicans* 20231). The different strains were obtained clinicaly at the Central Hospital of the University of Yaounde and the National Laboratory of Quality of Drugs and Expertise (LANACOM). Two standard drugs, Ciprofloxacin (Sigma, St. Louis, MO) and Amphotericin B (Sigma, St. Louis, MO), were used as reference antibiotics.

2.4.4. Preparation of the culture mediums

The different culture mediums prepared were the Muller Hinton Agar, Muller Hinton Broth, gelose de Sabouraud and Chloramphenicol and the Broth Sabouraud.

2.4.5. Preparation of the microbial inoculum

The Mueller Hinton Agar and the Sabaroud Dextrose Agar were firstly introduced in the petri dish. After solidification, they were seeded from the hold colonies, sealed with aluminium paper and incubated for 24h at 37 °C. The young colonies were later isolated for preparation of the inoculum. Two young colonies of 24h for bacteria and 48h for yeast were removed and introduced in a tube containing 10mL of steril physiological water (NaCl 0,9%), whose turbidity was compared to the standard 0,5 of Mac Farland or $1,5 \times 10^8$ UFC/mL for bacteria and $2,5 \times 10^6$ UFC/mL for yeast. The calibrated solution was diluted to obtain a microbial charge of 106 UFC/mL for the bacteria and 105 UFC/mL for the fungi.

2.4.6. Sensibility test of microorganism to extracts

The prepared inoculum was introduced on gelose Mueller Hinton broth or gelose Sabouraud supplemented with Chloramphenicol at 0.5% for the bacteria and yeast,

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respectively. Steril wells, incorporated with 10mg/mL of the extracts were incubated aerobically at 37 °C for 30 min and introduced in gelose medium. The tested concentrations for the extracts ranged from 10 mg/mL to 0,156 mg/mL. Wells of 5µg of Ciprofloxacin (for bacteria) and 20 µg of Amphotericin B (for yeast) were also introduced in the corresponding gelose medium, which was incubated at 37°C for 24h. The absence of microbial growth around each well revealed the antimicrobial or antifungal potency, shown by the measure of inhibition diameter. The sensibility of extracts was considered as follows: inhibitory diameter lower than 8mm, No sensibility (-); 9 to 14mm, sensible (+); 15 to 19mm, good sensibility and more than 20mm, very good sensibility (+++) (Igwe and Mgbemena, 2014).

2.4.7. Determination of antimicrobial parameters (MIC, MBC, MFC)

2.4.7.1. Determination of minimal inhibitory concentration (MIC)

The Minimum inhibitory concentration (MIC), which is regarded as the lowest concentration of sample that inhibits microbial growth or induces a colorimetric change from red to yellow as a result of the formation of acidic metabolites, signifying microbial growth (Carbonnelle et *al*., 1987; Berghe and Vlietinck, 1991), was determined using the 96-wells broth microdilution method as described by CLSI in 2012 (protocol M07-A9) with slight modifications (CLSI, 2012).

100 µL of the Mueller Hinton broth was introduced in each capsule of the microplate except for colons 3, representing the control (extract +MHB/SDB+ Chloramphenicol), 6 the sterilised control (MHB/SDB steril) and 8, the unused colons, in the operations. Six dilutions were done to obtained the concentration ranging from 10 to 0,156 mg/mL of plant extract in each plate. All experiments were carried out in duplicate. The refrences (Ciprofloxacin ou Amphotericin B) was submitted to the same procedure at concentration of 1 to 0,015mg/mL. And 100 µL of the inoculum were introduced in each capsul with the exception of the steril control plate incubated at 37 °C for 24h, with the turbidity visualy evaluated. The smallest concentration of extract from which no visible growth of bacteria/yeast was observed due to the absence of turbidity was considered as the Minimal inhibitory concentration.

2.4.7.2. Determination of the MBC and the MFC

150 µL of steril Mueller Hinton Broth or Sabouraud Dextrose Broth was introduced in the capsules of the microplate different form that of the MIC. 50 µL of the extracts were

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added to the capsules, where MIC were obtained. The steril control constituted of 200 µL of MHB/SDB medium. The reference was Ciprofloxacin et Amphotericin B.

2.4.8. DPPH radical scavenging assay

Stock solutions were prepared at 2 mg/mL in 100% ethanol; 0,02 % of DPPH solution was also prepared in ethanol and stored in absolute darkness at 4°C for 24h. 100 µg/mL of ascorbic acid, was used as reference. The anti-radical activity of the different extracts of *Peperomia pellucida* were evaluated using the protocol described by Dieng (2015), with slight modifications. 100 µL of ethanol and 100 µL of each extract were introduced in the 96 wells of the microplate; and 100 µL of $FeCl_3$ was added to yield the final concentration.

100µL of extract were introduce in line A and 100µL of ethanol in line B to H. Capsules from lines A to G were diluted to obtain the final concentration of extract ranging from 500 to 7,81 µg/mL. 100µL of DPPH at 0,02% was then added in each capsule. The negative control was DPPH and the positif control was the ascorbic acid with concentration ranging from 25 to 0,390625µg/mL. The tests were done in duplicate. After 60 min of incubation in dark at aerobic temperature, the optical density was mesure at 517 nm.

2.4.8.1. Determination of different parameters of scavenging DPPH radicals

The results of anti-radical or free radical inhibition activity are given in percentage using the following formular.

$$I(\%) = (Ao \times Ae)/Ao \times 100$$

Where, I (%): Inhibitory percentage;

$[A]_O$: Absorbance of negatif control;

$[A]_e$: Absorbance of extract.

The scavenging concentration (SC_{50}) or inhibitory concentration (IC_{50}) is the concentration of antioxidant which can scavenge 50% of free radicals. The effective concentration at 50% (EC_{50}) is the concentration of antioxidant which can reduce 50% of the initial concentration of DPPH.

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$$EC_{50} = SC_{50} / [\text{DPPH radical}]$$

EC_{50} : 50% Effective Concentration (µg/mL);

SC_{50}: Scavenging concentration (µg/mL);

[Radical DPPH] : Concentration of DPPH radical;

The anti-radical power (AP) is inversely proportional to the effective concentration at 50%. It measures the effectiveness of the anti-radical agent. PA= $1/EC_{50}$

2.4.8.2 Reduction of Fe (III) Ion

The Fe (III) ion reduction test was performed according to the protocol described by Path (1994), with sligth modifications. This method is based on the ability of a substance to reduce Fe^{3+} ions to Fe^{2+} ions. In the presence of 1,10-phenanthroline Fe^{3+} form a red-orange complex, whose optical density can be measured at 490 nm. The color intensity is proportional to the amount of Fe^{3+} ions converted by the extract.

2.4.8.3 Preparation of stock solutions

Extracts stock solutions were prepared at a concentration of 2mg/mL. 20mg of each extract, was weighed and dissolved in 10mL of ethanol. Fe^{3+} solution was prepared at 1.2 mg/mL. That is 1.2 mg of Ferric Chloride powder ($FeCl_3$) was dissolved in 1 mL of distilled water.

Ortho-Phenanthroline solution was prepared at 0.2%. This reagent was used to measure the amount of Fe^{2+} formed. 200 mg of each powder was weighed and dissolved in 100mL of ethanol. Vitamin C solution was prepared at 100µg/mL

2.4.8.4 Performing test

In a 96-well microplate, 100µL of ethanol was introduced into all the wells from line B to H (with H considered as a negative control). 100µL of each extract was added in duplicate in line A series of dilution of order 2, to line G ; followed by addition of 100µL solution of $FeCl_3$ to obtain concentrations of extracts of 500, 250, 125, 62.5, 31.25, 15.625 and 7.81µg/mL. The plates were incubated at room temperature for 15 minutes. After this incubation, 25 µL of the ortho-phenanthroline solution was added and the plates were re-incubated for 15 minutes. The optical densities of the contents of the wells were read at 490 nm, with a microplate reader spectrophotometer (Infinite model M200). The negative control

corresponding to 0% reduction consisted of 50 µL of ethanol, 50 µL Fe^{3+} and 25 µL of ortho-phenanthroline. The vitamin C, which served as a positive control was treated in a similar way to the extracts, with final concentrations ranging from 25 to 0.390625µg/mL. The results were expressed by the curve of variation of the optical density as a function of the concentration.

2.4.9. Statistical analysis

Curves were used for data comparison, were developped using Microsoft Excel version 2016 software with Windows version 10 professional. The STATGRAPHICS plus 5.0 version software was used to determine the inhibitory concentrations. The results obtained were expressed as means ± standard deviations. Probability values $P<0.05$ were considered statistically significant. The Waller Ducan test analysis (ANOVA) allowed us to compare more than two samples.

3. Results

3.1 Yields of different extractions

The information relating to the masses of the extracts, aspect and yields are given in Table 1

Table 1: Mass, yields and aspects of *P. pellucida* plant extracts

	AE	EtOH	EtOH/H_2O	HexE
Mass of powders (G)	34	34	34	34
Mass of extracts(G)	4	2,4	3	1,28
Yields (%)	11,7	7	8	3,76
Physical Aspect	Powdery	Pasty	Pasty	Sticky
Color	Light brown	Dark	Brown	Dark

AE: Aqueours extract; **EtOH:** Ethanolic Extract**;** **EtOH/H_2O:** Hydroethanolic extract; **HexE:** Hexane Extract

From the table, it appears that the yields vary from 3,76 % to 11,7 %. The ethanolic extract have the best yield. Concerning the aspects, it varied from powder to pasty and the colours from brown to dark.

3.2 Phytochemical Screening of different extracts

The chemicals compositions of the different extracts, evaluated by phytochemical screening are represented in table 2.

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Table2: Phytochemical screening results

Secondary metabolites	EtOH	EtOH / H_20	HexE	AE
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	-
Anthocyanins	-	-	-	-
Coumarins	+	-	+	-
Saponins	+	+	+	-
Tanins	+	+	+	-
Triterpenes	+	-	-	-
Steroids	-	+	+	-
Quinones	-	-	-	-

+: Presence; -: Absence; AE: Aqueous extract; **EtOH:** Ethanolic extract ; **EtOH/H_2O:** Hydroethanolic extract (70/30) (v/v); **HexE:** Hexane extract.

The results of the phytochemical screening revealed the presence of compounds such as phenols, flavonoids, alkaloids, coumarins, saponins, tanins, steroids, triterpenes and the absence of anthocyanes and quinones in the extracts. The aqueous extract contained less classes of secondary metabolites, compared to the others.

3.3 Antimicrobials Activities

3.3.1 Sensitive test of the extracts

The extracts were tested on their sensitivity to inhibit the growth of some microgorganisms. The results are shown in table 3.

Table 3: Results of the sensibility test of the different extracts

Bacteria	EtOH	EtOH/H_20	AE	HexE	Cp

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S. aureus ATCC 25923	+	-	++	+	++
K. Pneumoniae CHUY	-	-	-	-	-
P. aeruginosa ATCC 27853	-	-	++	++	+++
E.coli ATCC 35218	+	+	++	++	+++
S .enterica CHUY	+	+	++	++	+++
E ATCC 5199	-	-	+	+	+++
S .typhi CHUY	+	+	++	+++	+++
Fungal strains					Am
C. albicans ATCC 20231	+	-	-	+	++
C. CHUY	+	-	-	+	++

(-): Not sensitive; (+): sensitive; (++): good (+++): very sensitive; **AE:** Aqueous extract; **EtOH:** Ethanoilc extract**; EtOH/H_2O:** Hydroethanolic extract **(70/30) (v/v)**; **HexE:** Hexane extract **Cp**: Ciprofloxacin; **Am**: Amphotericin B; ***K***: *Klebsiella;* ***S****: Staphylococcus*; ***P***: *Pseudomonas*; ***E***: *Esherichia*; **E**: Enterococcus; **C**: *Candida*; **S**: *Salmonella*

The antimicrobial profil varied from insensitive (-) to very sensitive (++) with an inhibition diameter ranging from 0 to 16 mm. Table 3, showed that, hexane extract was the most active against the microbial and fungi strains. The aqeous extract inhibited the bacterias but was inactive on the fungi strains. The ethanolic extract demonstrated sensitivity on some of the bacteria and fungi strains. The least activity was exhibited by the hydroethanolic extract. All the extracts were inactive against *Klebsiella pneumoniae* CHUY.

3.3.2 Determination of the MIC, MBC and the reports MBC/MI C and MFC/MIC

Except for *Klebsiella pneumoniae* CHUY on which the extract was inactive, the MIC, MBC and MFC evaluated on the other strains are represented in table 4.

Table 4: MIC, MBC and MFC of different extracts

	EtOH		**EtOH/$H_2$0**		**AE**		**HexE**		**Cp/Am**	
	Antimicrobial parameters (mg/mL)									
Bacterial strains	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
S. aureus ATCC 25923	5	> 10	NT	NT	1,25	5	2,5	5	0,25	1
E. coli ATCC	NT	NT	1,25	10	5	> 10	1,25	10	0,015	0,015

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S. enterica CHUY	5	> 10	5	> 10	1,25	10	1,25	10	0,031	0,031
Sal Typhi CHUY	5	> 10	1,25	> 10	1,25	5	0,625	1,25	0,031	0,031
P. aeruginosa ATCC 27853	NT	NT	NT	NT	2,5	10	1,25	10	0,031	0,031
E .faecalis ATCC 5199	NT	NT	NT	NT	5	> 10	2,5	> 10	0 ,125	1
Fungal strains	**MIC**	**MFC**	**MIC**	**MFC**	**MI C**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
C. albicans 20231	2,5	5	NT	NT	NT	NT	2,5	10	0,031	0,031
C. Calbicans CHUY	2,5	5	NT	NT	NT	NT	2,5	10	0,031	0,031

NT: not tested **AE:** aqueous extract; **EtOH:** Ethanolic extract**; ETOH/H$_2$O: Hydro ethanolic extract (70/30) (v/v)** ; **HexE:** Hexane extract **Cp** : Ciprofloxacin ; **Am** : Amphotericin B ; ***K*** : *Klebsiella;* ***S*** *: Staphylococcus* ; ***P***: *Pseudomonas*; ***E*** *Esherichia* ; ***E*** *: Enterococcus*; ***C*****:** *Candida*; S : *Salmonella*; **> 10** : Greater than 10 mg/mL; Cp/Am : Ciprofloxacin / Amphotericin B

Table 4, indicated that, the hexane extract had bactericidal effect on *Salonela Typhi CHUY* with a MBC of 1.25 mg/mL, all the other extracs was bacteristatic on the tested bacteria strains. None of the tested extracs had fungicidal effect. As concerns the minimal inhibitory concentration, all the extracts were active on the bacteria and fungi strains with MIC values ranging from 5 to 0,625 mg/mL.

Table 5: The different concentrations MIC, MBC and MFC

	EtOH	EtOH/H$_2$0	AE	HexE	Cp/Am
Bacterial strains	**MBC/MIC**	**MBC/MIC**	**MBC/MIC**	**MBC/MIC**	**MBC/MIC**
***S. aureus* ATCC 25923**	ND	NT	4	4	4
***E. coli* ATCC 3521**	NT	8	ND	4	1
***S. enterica* CHUY**	ND	ND	8	8	1
S. Typhi	ND	ND	4	2	1
***P .aeruginosa* ATCC 27853**	NT	NT	4	8	1

***E. faecalis* ATCC 5199**	NT	NT	ND	ND	8
Fungal strains	**MFC/MIC**	**MFC/MIC**	**MFC/MIC**	**MFC/MIC**	**MFC/MIC**
***C. albicans* ATCC 20231**	2	NT	NT	4	1
C. Calbicans CHUY	2	NT	NT	4	1

AE: aqueous extract; **EtOH:** Ethanolic extract**; EtOH/H_2O: Hydro ethanolic extract (70/30) (v/v)**; **HexE:** Hexane extract **Cp**: Ciprofloxacin; **Am** : Amphotericin B; **Cp /Am**: Ciprofloxacin/Amphotericin B; ***K***: *Klebsiella; **S**: Staphylococcus*; ***P*** : *Pseudomonas* ; ***E*** : *Esherichia* ; ***E** : Enterococcus* ; ***C* :** *Candida*; S: *Salmonella*; **NT**: not tested; **ND** : not determined

Among the four extracts tested, the aqueous and hexane extracts showed activity against most of the strains with MIC values of 5 to 0,625 mg/mL.

Hexane extract demontrated stronger activity against the gram negative (*Salmonella typhi*), gram positive (*Staphylococcus aureus)* and fungi (*candida albicans*) with MIC values of 2,5 mg/mL.

The aqueous extract showed activity on the gram negative (*Pseudomonas aeruginosa* ATCC 27853 and *salmonella typhi*) and gram positive (*staphylococcus aureus*) bacteria strains ; while ethanolic extract presented no activity, though it was active against *candida albicans* with a MFC/MIC of 2, closer to that of Amphotricine B. The hydroethanolic extract was inactive against all the micrbial strains. Ciprofloxacin, the reference drug showed bactericidal activity on all the tested strains except for *Enterococcus faecalis* ATCC 5199 ; while Amphotericin exhibited fungicidal potency on all the strains.

3.4 Antioxidant Activity

3.4.1 Scavenging of free radical with DPPH

The anti radical activity was determined by evaluating the quality of the radicals trapped with respect to the concentration of the extract and the results obtained are

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represented on figure 1.

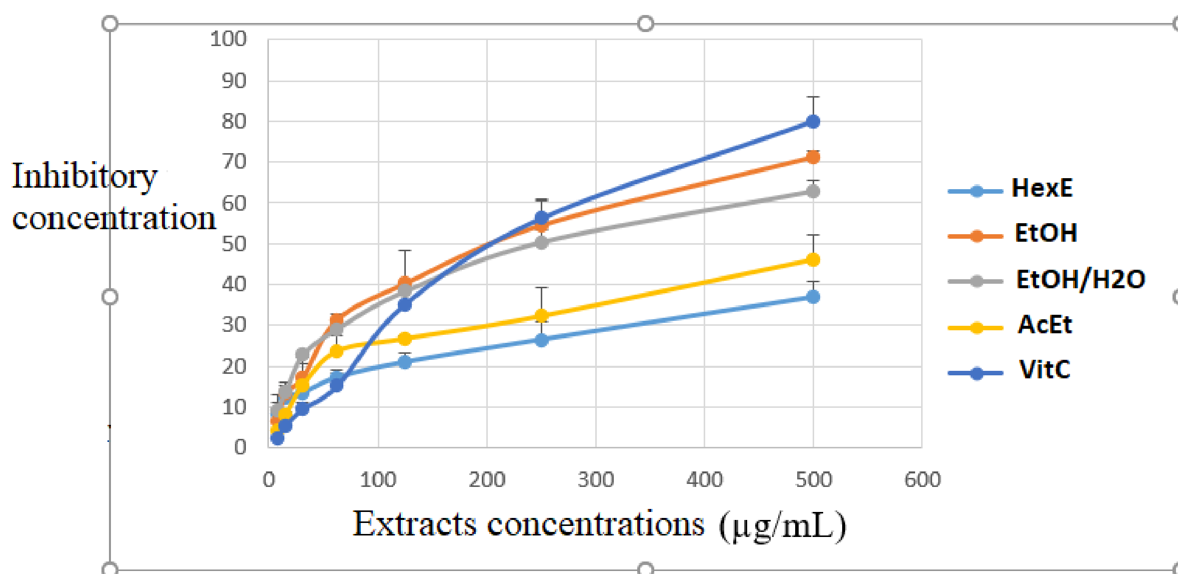

Figure 1: Yield of DPPH trapped with respect to the concentration of the extract

EtOH : Ethanoilic extract**; EtOH/H_2O:** Hydro ethanolic extrac (70/30) (v/v);
AE: Aqueous extract; **HexE:** Hexane extract; **Vit C**: vitamin C.

Figure 1, shows that the curves of the yields of DPPH trapped more than 50 % DPPH of ethanolic and hydroethanolic extractsl. From these percentages, the *Scavenging* concentration 50 (SC_{50}), the inhibition concentration 50 (IC_{50}) and the anti radical power are calculated and the results presented in table 6.

Table 6 : The IC_{50}, EC_{50}, AP of different extracts

Samples	**IC_{50} (µg/ml)**	**EC_{50} x 10^3 (µg/ml)**	**AP x 10^{-5} (µg/mol)**
HexE	/	ND	ND
EtOH	160,22 ± 4.509	10,681 ± 0.530	9,378 ± 0.466
EtOH/H_2O	245,08 ± 64.501	16,339 ± 0.043	6,120 ± 0.016
AE	/	ND	ND
Vit C	9,777 ± 0.520	0,652 ± 0.029	156,961 ± 29.959

EtOH : Ethanolic Extract **; EtOH/H_2O :** Hydro ethanolic extract (70/30) (v/v) ; **HexE :** Hexane extract ; **AE :** Aqueous extract; **Vit C :** Vitamin C

IC $_{50}$: inhibitory Concentration 50 ; **EC_{50}** = efficience Concentration 50 ; **PA** : anti radical power; / : IC_{50} > 500 **µg/ml** ; ND : not deduse

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Table 6 shows that the IC_{50} ranges from 160,22 µg/ml for the ethanolic extract to 245 µg/ml for the hydroethanolic. These values are less than that of Vitamin C (9,77 µg/ml) used as refference with a significant difference $p \geq 0,05$. Although the ethanolic extract had a scavenging activity lower than that of the vitamin C, it was more active than the hydroethanolic extract with an anti radical power of 9.378×10^{-5} g/mol.

3.4.2 Reduction of Fer III

The results of the variation of the optical densities with the concentration of the extract and vitamin C are reported in figure 2.

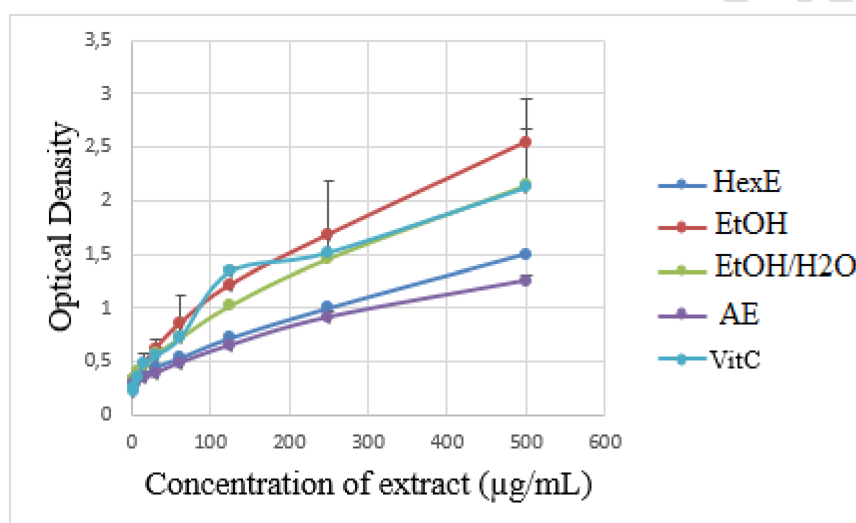

Figure 2 : Optical density of the extracts with their concentrations

EtOH: Ethanolic extract**; EOH/H_2O :** Hydroethanolic extract; **HexE:** Hexane extract; **AE :** Aqueous extract; **Vit C:** Vitamin C.

These results showed that, the ethanolic extract presented a good reduction power compared to the others. The optical density increased with the reduction of Fe^{3+} to Fe^{2+}. This was more than that of vitamin C.

4- Discussion and conclusion

The phytochemical screening of the hexane, ethanolic, hydroethanolic and aqueous extracts of *Peperomia pellucida* revealed the presence of alkaloids, coumarines, flavonoids and tanins. However, anthocyanins and quinones were absent, contradicting the results of

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Idris et *al.,* 2016, indicating the presence of these metabolites in *P. pellucida*. The difference could have been influenced by the place of havest, the period and the ecological factors.
The yield of the extracts ranged from 3,76 % to 11,7 %, with th**e** aqueous extract; having the greatest yield. These values are in accordance with those of Ibibia, 2012, who demonstrated that, the yield of the extraction of the leaves of *Peperomia pellucida* increased with the polarity of the solvent.

As concerns the antimicrobial test of the extracts on the nine strains at concentration of 10 mg/mL, only *Klebsiella pneumoniae* was inactive. This result is similar to that of Ibibia, 2012, who proved the insensibility of the hexane, methanolic and aqueous extract at concentration of 1mg/mL on *Klebsiella pneumoniae*.

The hydroethanolic extract showed weak activity against the bacteria and fungal and was bacteriostatic on *E. coli* ATCC 35218, while the ethanolic extract demostrated good activities on both the bacteria and fungi strains, with MIC values of 2,5 - 5 mg/mL. The MFC/MIC of the ehtnolic extract had a value of 2. The aqueous extract, showed a strong activity against the gram negative bacterias (*Salmonella typhi* and *Speudomonas aeruginosa* ATCC 27853), exhibiting a bactericidal effect. According to several studies, this selectivity could be expressed by the differences in parietal structures of the two groups. The wall of the gram negative bacteria, rich in lipids, would facilitate the penetration of hydrophobic molecules, while the gram positiv bacteria, rich in teichoic acids favors the entry of negative charge molecules.

The hexane extract was the most active of all, it had activity not only against gram positive (*Staphylococcus aureus* ATCC 25923) and negative (*Salmonella typhi, Pseudomonas aeruginosa* ATCC 27853 et *Escherichia coli)* bacteria, but also against *candida albicans* with MIC ranging from 0,625 - 5 mg/mL. This is in accordance with the results of Zubair, 2015, who indicated that, the hexane extract of *Peperomia pellucida,* shown higher antimicrobial activity than those of the aqueous and chloroform extracts against *Streptococcus agalactiae, Salmonella typhi, Escherichia coli* and *Proteus mirabilis.* The effectiveness of this extract could be due to its high polyphenolic and flavonoid content (Zubair, 2015)

As concerns the antioxidant potency of the extracts using DPPH, the highest activity was observed in the ethanolic and hydroethanolic extracts with IC_{50} values between 160,22 ± 4,509 and 245,08 ± 64,501 (µg/mL), respectively. The high activity of these extracts could be

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due to the presence of phenols, tannins and flavonoids. In fact, these secondary metabolites have the ability to trap free radicals through their OH groups (Zubair, 2015). The ethanolic extract presented a strong reducing power, characterized by the increase of the optical density as compared to the reference (Vitamin C). This could be due to the prensence of phenolics and their ability to give up electrons to Fe^{3+}, thereby reducing it to Fe^{2+} (Zubair, 2015).

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