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The phytochemical profile, anti-microbial and anti-oxidant properties of *pentas schimperiana* (*rubiaceae*)

Profil phytochimique, propriétés anti-microbiennes et antioxydantes de pentas schimperiana (rubiaceae)

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Article original

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ABSTRACT

Introduction: Antimicrobial resistance is a growing concern worldwide. The aim of our study was to assess the antimicrobial and anti-oxidant activity against bacteria and their potential as sources of *Pentas schimperiana.*
Materials and Methods: The experimental study was carried out to evaluate the antimicrobial and anti-oxidant potential of *Pentas schimperiana* plant extracts against five pathogenic multi-resistant bacterial strains obtained from clinical isolates and gallic acid for anti-oxidant. The extracts were fractionated using liquid-liquid separation. The antimicrobial activity was carried out *in vitro* by Broth micro-dilution method, with Ciprofloxacin as reference antibiotic.
Results: The quantity of secondary metabolites in decreasing order of concentration were present in the methanolic crude extract of plant with values of Polyphenols 200µg Eca/mg MS, alkaloid 180µg Eca/mg MS, terpenoids 120 µg Eca/mg MS, Saponins 50 µg Eca/mg MS, flavonoids 35 µg Eca/mg MS, coumarins 10µg Eca/mg MS. The results of the micro-dilution assay showed that *Pentas schimperiana* plant extracts exhibited significant antimicrobial activity against all the bacteria strains. The MIC values ranged from 500 µg/ml to 1000 µg/ml, while no MBC values were recorded at concentrations ≤ 1000µg/ml showing that they were bacteriostatic. For the anti-oxidant activity, the extract showed activity in trapping the DPPH radical with an IC50 of 26.7 µg/ml crude extract which was the most significant and reducing ferric iron to ferrous iron at different concentrations.
Conclusion*: Pentas schimperiana* plant extracts exhibit antimicrobial and antioxidant activities. This plant would be a potential source for the treatment of many diseases.

RESUME

Introduction : La résistance aux antimicrobiens est une préoccupation croissante dans le monde entier. Le but de l'étude était d'évaluer la potentielle activité antimicrobienne et antioxydante de *Pentas schimperiana.*
Matériel et Méthodes : Une étude expérimentale a été réalisée pour évaluer le potentiel antimicrobien et antioxydant des extraits de plantes Pentas schimperiana sur cinq souches bactériennes pathogènes multirésistantes. Les extraits ont été fractionnés par séparation liquide-liquide. L'activité antimicrobienne a été réalisée in vitro par la méthode de micro-dilution en bouillon, avec la ciprofloxacine comme antibiotique de référence.
Résultats : La quantité de métabolites secondaires par ordre décroissant de concentration était présente dans l'extrait brut méthanolique de la plante avec pour valeurs de Polyphénols 200µg Eca/mg MS, alcaloïdes 180µg Eca/mg MS, terpénoïdes 120µg Eca/mg MS, Saponines 50 µg Eca/mg MS, flavonoïdes 35µg Eca/mg MS, coumarines 10µg Eca/mg MS. Les extraits de la plante de Pentas schimperiana présentaient une activité antimicrobienne significative contre toutes les souches bactériennes et étaient bactériostatiques. Les valeurs de CMI variaient de 500 µg/ml à 1 000 µg/ml, alors qu'aucune valeur de MBC n'a été enregistrée à des concentrations ≤ 1 000 µg/ml. Pour l'activité antioxydante, l'extrait a montré une activité de piégeage du radical DPPH avec un CI50 de 26,7 µg/ml d'extrait brut qui était le plus significatif et réduisait le fer ferrique en fer ferreux.
Conclusion : Les extraits de plantes *Pentas schimperiana* présentent des activités antimicrobiennes et antioxydantes. Cette plante serait une source potentielle pour le traitement de nombreuses maladies.

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Introduction

For thousands of years, plants constitute a vast biodiversity of vegetation throughout the world, in Africa and particularly in Cameroon. Faced with the limitations of modern medicine, socio-cultural habits and poverty, African populations are turning to traditional medicine, which is proving to be effective for human health [1]. According to a 2020 report by the Centre for Disease Dynamics, Economics and Policy (CDDEP), globally, around 700 000 people die each year due to antimicrobial resistance. The report also states that the economic impact of antimicrobial resistance could be up to 100 trillion dollars by 2050 [2]. In Africa, a 2021 study by Iwu-Juju *et al*. found that the prevalence of antibiotic resistance in bacterial infections was high, with resistance rates ranging from 32.5% to 96.8%. The study also highlighted the need for increased efforts to control and prevent the spread of antibiotic resistance in Africa [2]. The emergence and spread of drug-resistant pathogens is leading to the acquisition of new resistance mechanisms, leading to antimicrobial resistance and continue to threaten our ability to treat common infections. The rapid global spread of multi-resistant and pan-resistant bacteria, also known as "superbugs", which cause infections that cannot be treated infections with existing antimicrobials such as antibiotics, is of particular concern.

Oxidative stress corresponds to an imbalance between the production of activated oxygen species (AOS) and the body's antioxidant defences. This exposes us to various pathologies such as cancer and cardiovascular disease [3]. The use of synthetic antioxidant molecules is currently being questioned toxicological risks. New sources of natural sources of natural antioxidants [4, 5]. Furthermore, unfavourable conditions for plants, such as extreme temperature, drought, heavy metals, nutrient deficiencies, and high salinity, generate high concentrations of reactive oxygen species (ROS), which can cause oxidative stress. To avoid this, cells have a complex antioxidant system with enzymatic and non-enzymatic elements. The molecules of the non-enzymatic system have different action mechanisms, such as enzyme inhibition, chelation of trace elements involved in the production of free radicals, reactive species uptake and activation or increase in protection through other antioxidant defenses [6]. The choice of this plant was motivated on one hand by its traditional used in Ethiopian traditional medicine for the treatment of epilepsy, diabetes and bacterial infections and the other hand the previous pharmacological studies shows that the plant has antidiabetic and anticonvulsant properties [7,8]. we propose to study and evaluate the anti-microbial and antioxidant activities of the methanolic extract of *Pentas schimperiana.* [7].

Material and Methods

Phytochemical

Plant material

Pentas schimperiana was collected in the Southwest Region of Cameroon more precisely in the fako division. Its habitat is in Buea at the mount Cameroon with an altitude of 2200m that is a Bokwango area growing in the savannah. It has as latitude 4 degree 08'N longitude 9 degree 07'E.

Figure 1. *Pentas schimperiana plant in natural habitat*
Source: picture of Tse Hanley (Buea /Cameron/2023)

After drying, a mass of plant will be obtained. It was crushed using a homemade machine and all these fibers are then macerated in 10 l of methanol for 3 days (72 h) at room temperature, with occasional stirring to increase extraction yield. Following maceration, the extract will be filtered through wattman No. 4 filter paper with a pore size of 0.45 mm and finally concentrated at 96°C using a rotary evaporator. After drying the extract, a thick brown, oily crude is obtained. Finally, some of crude extract will be set aside for phytochemical screening, some for *in vitro* testing and the rest for liquid-liquid separation. Double maceration of 4.7 kg of the crushed plant in 90% methanol (for 72 h) yielded crude extracts of 350.341g after filtration and evaporation using a rotary evaporator. Yields were calculated.

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

Liquid-liquid separation

Fractionation by liquid-liquid extraction is a separation method based on0 the difference in solubility of a solute in two immiscible phases. In this work, an aqueous fixed phase and a mobile phase consisting of organic solvents of increasing polarities (Hexane, dichloromethane, and ethyl acetate) were used. After evaporation under reduced pressure using a rotary evaporator, we obtained 350 g of crude plant extract of *P. schimperiana*, from which we withdrew 50 g per extract for phytochemical screening, evaluation of anti-bacterial, anti-fungal and anti-oxidant activity. The remaining quantities were used to perform liquid-liquid extractions with different solvents of increasing polarity, using a separating funnel according to the following procedure. The crude extracts were dissolved separately in 200 ml distilled water; the various solvents (hexane-HE, dichloromethane-DCM, and ethyl acetate-ACE) were added at a rate of 200ml, followed by decantation to collect the crude extract fraction. This operation was repeated several times to increase the yield.

Antimicrobial activity of the plant extracts

The microorganisms used in this study were multi-drug resistant bacteria strains which includes; *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, Acinetobacter baumanii*, *Proteus mirabilis* and fungal strains which where *Candida krusei* and *Candida glabrata.*

Preparation of culture media

The different culture media used in this work was prepared following the recommendations of the different manufacturers as follows [9].

- **Culture Medium:** Mueller Hinton Agar
  - **Uses**: Recommended for determination of susceptibility of microorganisms to antimicrobial agents.
  - **Method of preparation**: 38.0 g was suspended into 1000 ml distilled water was heated for 15 minutes and sterilized by autoclaving.
- **Culture Medium:** Muller Hinton Broth
  - **Uses:** Recommended to determine the susceptibility of bacteria by the tube dilution method.
  - **Method of preparation:** 21.0 g was suspended into 1000 ml distilled water was heated for 15 minutes and sterilized by autoclaving.

Culturing of microbial strains

The microorganisms used in this study were multi-drug resistant bacteria strains which includes; *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, Acinetobacter baumanii*, *Proteus mirabilis*. All samples were cultured and sub-cultured again to obtain pure colonies on their specific culture media in Petri dishes by quadrant method and incubated at 37^{o}C as recommended by the CLSI.

Preparation of McFarland standard

McFarland Standards are used to standardize the approximate number of bacteria or fungi in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of Barium Chloride and Sulphuric acid, the reaction between these two chemicals results in the production of a fine precipitate, Barium sulphate. When shaken well, the turbidity of a McFarland Standard is visually comparable to a bacterial or fungal suspension of known concentration. In order to perform the antimicrobial activity and evaluate the MIC and FICI, 0.5 McFarland Standard was prepared. It was chosen because it is the recommended standard suspension for antimicrobial susceptibility testing and culture media performance testing.

- **Procedure**

- 9.95 ml 1% H2.SO4. was pipetted and transferred into a test tube.
- 0.05 ml 1% BaCl2 was pipetted and added into the tube containing the H2SO4.
- The mixture was shaken vigorously and the turbidity observe
- Once prepared, the tube was tightly sealed to prevent evaporation from occurring
  The tube was labelled and stored in an upright position at room temperature away from light [10].

Preparation of inoculum

The inoculum suspension was prepared in the sterility zone around the blue flame by selecting several morphologically similar colonies from overnight growth (18–24 h of incubation) with a sterile loop. In order to obtain 24 hours old colonies, inoculum of each test strain was made and streaked on their respective culture media a day before by

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

quadrant method. The 24 hours' colonies were then suspended in sterile normal saline (0.9% NaCl w/v in water) and the density compared to that of a McFarland 0.5 standard which is equivalent to 1.5 x 10^8 CFU/ml for bacteria.

Preparation of the stock solution

In a sterile vial, our stock suspension was prepared at a concentration of 10mg/ml by dissolving 10mg of our extract in 1ml of DMSO **(**diméthylsulfoxyde). The antimicrobial activity of the extracts was assessed by determining the inhibitory parameters which are the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the Broth Micro dilution method as described by CLSI with minor modification [11].

Determination of the minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation. The method used to evaluate the sensitivity of bacteria our extracts is that of micro-dilution described in ISO 20776-1, 2006 and amended by the Antibiogram Committee of the French Society of Microbiology (CA- SFM/EUCAST 2019) [12]. The MIC was determined using the Broth micro dilution method which is the standard method recommended internationally for Antibiotic Susceptibility Testing. The MIC of the plant extracts and the reference drug (ciprofloxacin) were performed using a modified procedure described by Tenover [13]. The MIC was performed in duplicates using Sterile 96-well microliter plates.

- **Procedure**

- Stock solution of the antimicrobial agent and reference drug was prepared at a concentration of 800 µg/ml.
- Two-fold serial dilutions of the extract and reference drug (ceftriaxone) were prepared directly on 96-well microliter plate containing growth medium Mueller Hinton broth to obtain various concentrations ranging from 3.125µg/ml to 400µg/ml.
- Each well was inoculated with 50µl of freshly prepared 3 x 10^6CFU/ml inoculum. The positive control used was ceftriaxone dosed at the same concentration, the negative control containing culture medium and inoculum only.
- The plate was covered with a sterile sealer and incubated overnight at 37^0C. Resazurin was later added in each well of the microliter plate and was incubated at 37^0C for 30min.
-

Determination of the minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is defined as is the lowest concentration of an antibacterial or antimicrobial agent that kills a particular bacterium or reduces its viability by ≥99.9% after incubation for 72 hours [13]. The MBC was performed using a modified procedure from Santos [14]. Broth micro dilution method was used to examine the MBC for the antimicrobials. The MBC was performed from the MIC assay. A sterile 96-well plate was used.

- **Procedure**

- 50µl of samples were collected from wells with no visible growth in the MIC assay and introduced into microliter plate containing 150µl of MHB.
- The plate was covered with a sterile sealer and incubated for 72h at 37^0C. Resazurin was later added in each well of the microliter plate and was incubated at 37^0C for 30min.

in vitro antioxidant assays of plant extracts

Scavenging effect on DPPH (2,2-Diphenyl-1-picrylhydrazyl)

Radical: The scavenging effect of the extracts was determined using the protocol previously described [13]. Briefly, 25 µl of extracts prepared at concentrations of 1000,500, 250, 125 and 62.5 µg/ml was added to 75 µl of methanol solution of DPPH (0.02%) to obtain final volumes of 100 µl and final concentrations of 250, 125, 62.5, 31.25 and 15.625 µg/ml. Gallic acid prepared at an initial concentration of 1 mg/ml was used as a positive control. After 30 minutes of incubation in absolute darkness, the absorbance was read at 517 nm. Each experiment was performed in triplicate, and the percentage of inhibition of endophytic fungal extracts was calculated using the following equation: **RSA = (Ao - As)/Ao × 100**

Where RSA: Radical Scavenging Activity; Ao: Absorbance of the blank (DPPH + methanol); As: Absorbance of DPPH Radical + plant extract. From %RSA, other parameters, such as the RSA50, EC50, and ARP, were deduced.

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

RSA50 is the concentration of extract at which 50% of the free radicals are scavenged and is obtained from a graph of % RSA as a function of the logarithmic values of extract concentrations EC50 the efficient concentration, defined as the concentration of extract required to scavenge ½ mole of DPPH, was calculated as follows:
EC50 = RSA50/ [DPPH]

ARP; Antiradical power is the inverse of the EC50. It measures the efficiency of the antiradical; hence, the larger the ARP is, the more efficient the antiradical.
ARP = 1/ [EC50]

Ferric ion reducing antioxidant power (FRAP) assay

The assay was performed according to the method described by Yefrida *et al.* with slight modifications [14]. Briefly, 25 µl of each test sample was prepared at different concentrations (6.25, 12.5, 25, and 50 µg/ml) in the test plates, and 25 µl of iron (III) chloride (1.2 mg/ml) was added to the samples. Vitamin C served as positive control. Plates were incubated at room temperature for 15 minutes. After incubation, 50 µl of 1,10-phenanthroline (0.02%) was added, and then the absorbance of the mixture was determined at 517 nm through a spectrophotometer. The control contained iron (III) chloride, distilled water and 1,10-phenanthroline. Each experiment was performed in triplicate, and the reducing capacity of the endophytic fungal extracts was calculated using the following formula:
Reducing Fe^{3+} (%) = 1 – As/Ao × 100

Where Ao is the absorbance of the control (Fe^{2+} + ortho-phenanthroline) and as is the absorbance of the test (Fe^{3+} + extract + ortho-phenanthroline). From dose-response curves obtained from different concentrations of the samples, the concentration of sample required to scavenge 50% of Fe^{3+} (50% inhibition concentration, IC50) was determined.

Results

The harvested 12,3kg of fresh plant. It was wash and dried for 7 days under shadow and was later crushed and gave us a yield of 4,7kg. After carrying out cold maceration with methanol, great yields were obtained from the plant extracts of *P. schimperiana* with a yield of 7.7%. In addition, the physical characteristics such as the color and appearance of our extract were noted. An extraction followed by fractionation, phytochemical screening and evaluation of anti-bacterial, anti-fungal, anti-oxidant activity was done. The crude extracts obtained were fractionated by liquid-liquid extraction using distilled water as the fixed phase and a variant mobile phase consisting of the solvents of increasing polarity: hexane, dichloromethane and ethyl acetate. We thus obtained 4 dark-brown fractions (**Figure 2**) (aqueous fraction-Faq, hexane fraction-FH, ethyl acetate fraction-FA, dichloromethane fraction-FD).

Legend: *HFPS = Hexane fraction of Pentas schimperiana; DFPS = Dichloromethane fraction of Pentas schimperiana; AFPS = Aqueous fraction of Pentas schimperiana; EFPS = Ethyl-acetate fraction of Pentas schimperiana.*
Figure 2. Yield of the liquid-liquid fractionation

Phytochemical screening of the crude extracts

Overall, the phytochemical screening of *P. schimperiana* plant extracts (**Table I**) showed promising results for alkaloids, flavonoids, phenols,

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

tannins, terpenes, and steroids with small traces of saponins, phenols and alkaloids where more present in the different extracts than the others metabolites. The aqueous fraction of the plant (AFPS) and the crude extract of the plant (CFPS) from *P. schimperiana* contain more secondary metabolites than the other samples. Alkaloids and polyphenols are the metabolites most present in the samples. Saponins were found only in the crude extract of the plant (CFPS) of *P. schimperiana*.

Table I: phytochemical screening results of the different fractions of *Pentas schimperiana*

Metabolite	EFPS	AFPS	CFPS	DFPS	HFPS
Alkaloids	+	+	+	+	+
Flavonoids	-	+	+	-	-
Phenols	+	+	+	+	+
Coumarins	+	+	+	+	+
Saponins	-	-	+	-	-
Tannins	+	+	+	+	+
Terpenes	+	+	+	+	+
Steroids	+	+	+	+	+

Key:-=*Absent* + = *Present;* HFPS= Hexane fraction of *Pentas schimperiana*; DFPS= Dichloromethane fraction of *Pentas schimperiana*; AFPS= Aqueous fraction of *Pentas schimperiana*; EFPS= Ethyl-acetate fraction of *Pentas schimperiana;* CFPS= Crude fraction of *Pentas schimperiana*

Indication of linear equation and correlation coefficient

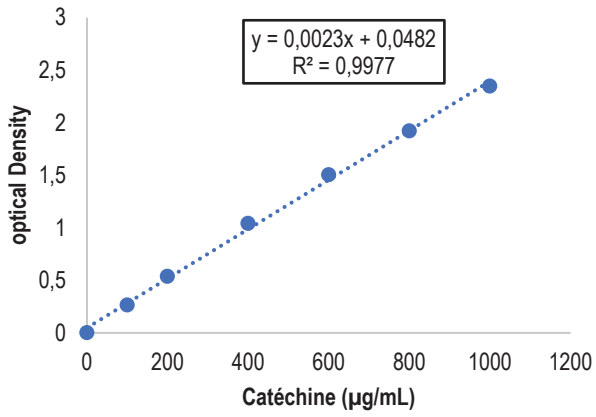


Figure 3: *polyphénols dosage calibration curve*

Quantitative extract screening results

The quantitative analysis (**Figure 6**) show that the quantity of polyphenols, alkaloids, terpenoid, saponins, flavonoids, coumarins, tannins in decreasing order of concentration where present.

The results of quantitative screening of crude extract are shown below.

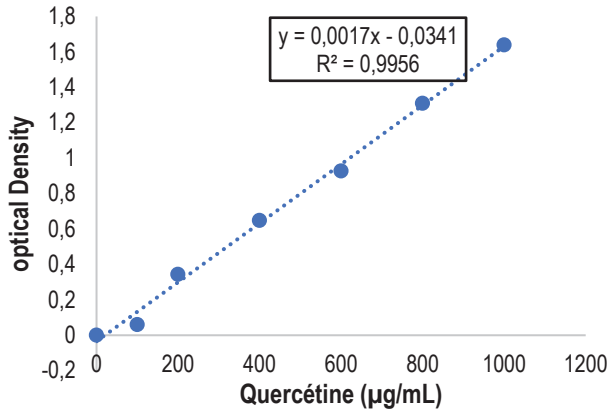


Figure 4: *flavonoïdes dosage calibration curve*

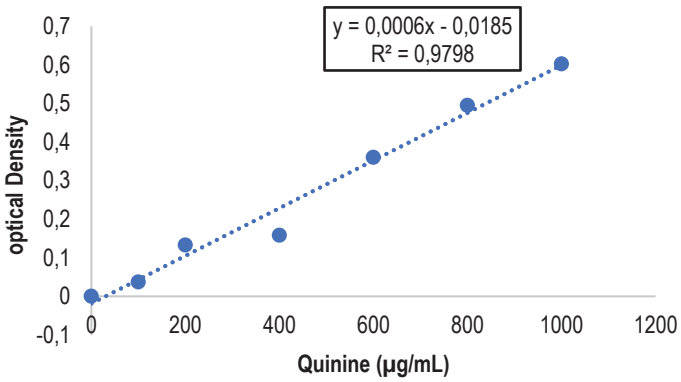


Figure 5: *alkaloid dosage calibration curve*

Antimicrobial activities

Antibacterial activity

The Table II Following the strains that exhibited sensitivity, the minimum inhibitory concentrations, the minimum microbicidal concentrations, and the ratio MMC/MIC of the total extract and of the various fractions of *P. schimperiana* concerned at a concentration of 1mg/ml were carried out by microdilution and the results are recorded in below shows the MIC of the plant extracts against different bacteria strains. All the plant extracts were active on all the bacteria strains with *P. schimperiana* showing a significant activity of the different fractions at 500 µg/ml to 1000 µg/ml on *K. pneumonaie, P. aeruginosa, E. coli* MDR1, *A. baumanii, P. mirabilis* compared to Ciprofloxacin and Gentamycin. ////9999/

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

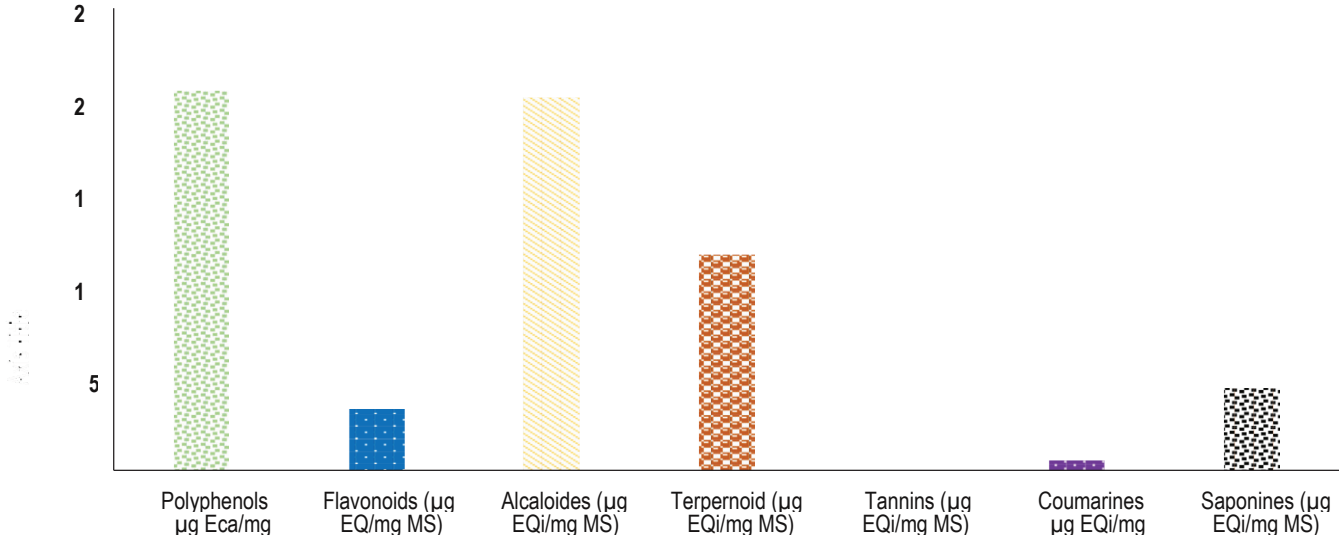


Figure 6. *Quantities of secondary metabolites found on the crude extract*

Table II: the MIC of the different extracts on the bacterials

Bacteria	State	Hexane extract (µg/ml)	DCM extract (µg/ml)	Acetate extract (µg/ml)	Aqueous extract (µg/ml)	Crude extract (µg/ml)	Cipro (µg/ml)	Gentamycin (µg/ml)
Klebsiella pneumonaie	MIC	500	1000	1000	1000	1000	100	100
	MBC	2500	2500	2500	2500	2500		
	MBC/MIC	5	2.5	2.5	2.5	2.5		
Pseudomonas aeruginosa	MIC	500	1000	1000	1000	500	100	100
	MBC	2500	2500	2500	2500	2500		
	MBC/MIC	5	2.5	2.5	2.5	5		
Escherichi coli MDR1	MIC	500	1000	1000	1000	500	100	100
	MBC	2500	2500	2500	2500	2500		
	MBC/MIC	5	2.5	2.5	2.5	5		
Acinetobacter baumanii	MIC	1000	1000	1000	1000	1000	100	100
	MBC	2500	2500	2500	2500	2500		
	MBC/MIC	2.5	2.5	2.5	2.5	2.5		
Priteus mirabilis	MIC	1000	1000	1000	1000	1000	100	100
	MBC	2500	2500	2500	2500	2500		
S	MBC/MIC	2.5	2.5	2.5	2.5	2.5		

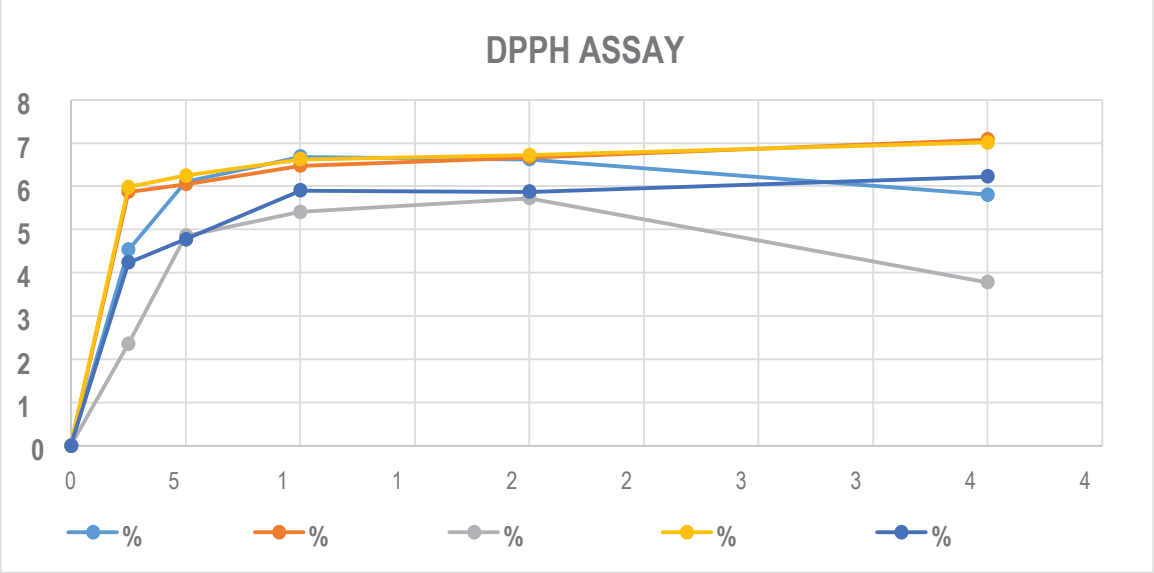
Table III: *the MIC of the different extracts on the fungals*

Fungi	State	Hexane extract (µg/ml)	DCM extract (µg/ml)	Acetate extract (µg/ml)	Aqueous extract (µg/ml)	Crude extract (µg/ml)	FUCLO
CG	MIC	500	1000	500	1000	500	100
	MFC	2500	2500	2500	2500	2500	
	MFC/MIC	5	2.5	5	2.5	5	
CK	MIC	1000	1000	500	1000	500	100
	MFC	2500	2500	2500	2500	2500	
	MFC/MIC	2.5	2.5	5	2.5	5	

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

Antifungal activity

This activity shows the MIC of the plant extracts against some fungal strains (**Table III**). All the plant extracts were active on all the fungal strains with *P. schimperiana* showing a significant activity at 500 µg/ml to 1000 µg/ml on *Candida* krusei and *Candida glabrata*. *P. schimperiana* extract showed a more significant activity at 500 µg/ml with the Hexane, Acetate, and Crude extract on *Candida glabrata* compared to flucoxacillin while a more significant activity at 500 µg/ml with the Acetate and Crude extract on *Candida* krusei compared to flucoxacillin.



***Legend**: DCM RSA= Racical scaving absorbtion dichloromethane extract, Racical scaving absorbtion crude extract, Racical scaving absorbtion hexane extract, Racical scaving absorbtion aqueous extract, Racical scaving absorbtion acetate extract.*

Figure 7: *inhibition percentages of the different extract*

Anti-oxidant activity

Activity on the 2,2-diphenyl-1-pycrylhydrazyl (DPPH) free radical

The figure below shows the evolution of the percentage inhibition as a function of the Concentration of extract and Gallic Acid.

The antioxidant activity of methanolic extract of *P. schimperiana* and the standard antioxidant standard antioxidant (Gallic acid) against the DPPH radical was evaluated using a spectrophotometer (**Figure 7**), following the reduction of this radical, which is accompanied by its from violet (DPPH-) to yellow (DPPH-H), measurable at 517 nm. This reduction capacity is determined by a decrease in absorbance induced by anti-free radical substances.Thus, a compound's anti-oxidant capacity is all the higher when the IC_{50} smaller. The figure below shows the IC_{50} values for Gallic acid and methanolic extract of *P. schimperiana.* The results show inhibition of the DPPH radical by Gallic acid and methanolic extract of *P. schimperiana* as a function of concentration, with IC_{50} values of 5.06 and 26.7 µg/ml respectively.

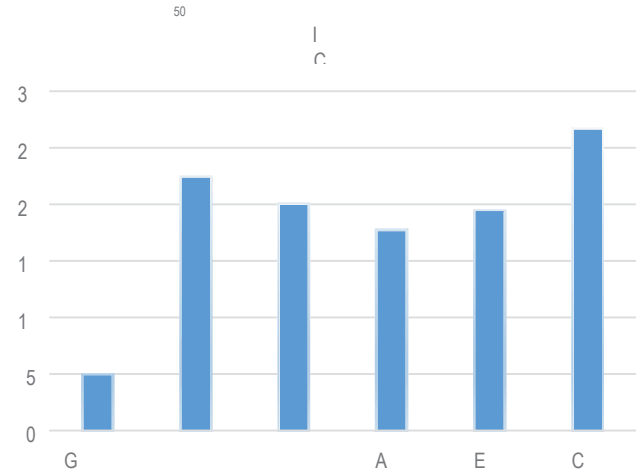


Figure 8: values of the IC_{50} of Gallic acid and the different methanolic extracts of *Pentas schimperiana*

Ferric reducing power

The ferric reducing power of the methanolic extract of *P. schimperiana is* shown in the figure below. The

Citation as **J Sci Dis 2025, Vol 3 (1): 44-53**

results show an increasing reducing power of Fe^{3+} to Fe^{2+} as a function of extract concentrations.

Discussion

The yields resulting from this fractionation showed that the aqueous fraction (AFPS) had the highest yield with a value of (32.4%), followed by the dichloromethane fraction (DFPS) with (3.2%), then the ethyl acetate fraction (EFPS) with (2.2%), then the Hexane fraction (EAFEl) with (1.06%) relatively to the total extract (ETFEl). These values would mean that *P. schimperiana* contain more polar because the greatest amount of the fractions are in polar solvents. At observing the extraction yields of *P. lanceolata* roots by Bukuru et *al*. different solvents (hexane (0.21%), dichloromethane (0.64%), ethyl acetate (1.07%), methanol (4.26%) [15], suggesting that the extraction efficiency of the roots of the species of this genus depended on the increasing polarity of the solvents used. This probably explains why the aqueous extraction performed had a higher yield; the decoction just seemed to accelerate extraction.
Although absent in some samples during qualitative analysis, alkaloids, polyphenols, flavonoids and tannins were found in all crude extracts and fractions using absorption spectrophotometry (quantitative analysis), confirming the sensitivity of this technique through its high detection threshold. Saponins were absent in some samples. A high alkaloid and polyphenol content relative to other metabolites was noted across all sample types. In the review we can see that the anti-microbial activity is caused by the presence of flavonoid, phenol, alkaloid while the anti-oxidant activities are due to the presence of terpenoids, sterols [16]

Phytochemical screening revealed the following in the methanolic extract of the stems alkaloids, saponins, phenols, flavonoids, bound quinones, triterpenes and sterols. In the methanolic extract of the roots, he found alkaloids, saponins, phenols, flavonoids, triterpenes and sterols, phenols, flavonoids, free quinones and sterols. The aqueous extract showed the same chemical classes as the methanolic root extract for free quinones, but rather bound quinones and, in addition, coumarins. Literature data confirm the presence of phenolic compounds, including anthraquinones, which are among the chemical classes most commonly present in the *Pentas* genus, as well as sterols and alkaloids [15]. Otherwise, saponins have been identified in *P. lanceolata* leaves [15].

According to Kuete in 2023, for plant extracts, we can have outstanding activity when MIC ≤ 8 µg/ml; excellent activity when 8 < MIC ≤ 64 µg/ml; very good activity when 64 < MIC ≤ 128 µg/ml; good activity when 128 < MIC ≤ 256 µg/ml, average activity when 256 < MIC ≤ 512 µg/ml, weak activity when 512 < MIC ≤ 1024 µg/ml, and not active MIC > 1024 µg/ml.

From this classification, total extract and different fractions are not active on different strains because the MICs values are > 1024 µg/ml [17]. According to the antimicrobial results obtain, the hexane fraction had an average activity while the dichloromethane fraction, acetate fraction, aqueous fraction, crude fraction had a weak activity when tested on *klebsiella pneumoniae* species. Hexane and crude fraction had an average activity while the dichloromethane fraction, acetate fraction, aqueous fraction had a weak activity when tested on *Pseudomonas aeruginosa and Escherichi coli*. All the extracts were weak when tested on *Acinetobacter baumanii* and *Priteus mirabilis*. The crude extract and the hexane fraction had the lowest MIC value effect on the different bacterial strains and would be considered the most active with respect to antimicrobial activity. Since the crude extract and hexane fraction is more active than the different fractions, it could be assumed that there are more active metabolites in these fractions.

According to Marmonier et *al*. [18] in 1990 when the ratio CMM/CMI < 4, the extract is considered microbicidal and if CMM/CMI ≥ 4, the extract is considered microstatic. It appears that the hexane fraction is microstatic since it has CMM/CMI ≥ 4 while the dichloromethane fraction, acetate fraction, aqueous fraction and crude fraction are microbicidal since CMM/CMI < 4 when tested on *Klebsiella pneumoniae* species. Hexane and crude fraction are microstatic since it has CMM/CMI ≥ 4 while the dichloromethane fraction, acetate fraction, aqueous fraction are microbicidal since CMM/CMI < 4 when tested on *Pseudomonas aeruginosa and Escherichi coli MDR1*. All the extracts microbicidal since CMM/CMI < 4 when tested on *Acinetobacter baumanii* and *Priteus mirabilis*. The extract showed antioxidant activity towards DPPH with an IC_{50} of 5.06 µg/ml, and at 500 µg/ml a concentration of 142.86 µg/g

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AG was able to reduce ferric iron to ferrous iron.

Conclusion

The assessment of natural products in relation to drug discovery was the goal of the present research. Due to the above, we underlined the antibacterial of *Pentas schimperiana* plant extract to examine their importance in comparison to a reference antibiotic ciprofloxacin. evaluate *in-vitro* the inhibitory parameters of the extracts that is the MIC and MBC and evaluate the anti-oxidant activity of the plant extract using DPPH and FRAP methods on the plant extract and fractions. The phytochemical screening of the extracts revealed the presence of polyphenols, alkaloid, terpenoids, saponins, flavonoids, coumarins, tannins in decreasing order of concentration where present. Alkaloids, flavonoids, Terpenes and Phenols are secondary metabolites responsible for anti-oxidant activity while terpene, sterol and tannins are responsible for anti-microbial activity. Regarding the *in-vitro* antimicrobial activity, we have been able to demonstrate that the plant extracts of *Pentas schimperiana* have comparative antibacterial property to that of the reference compound ciprofloxacin. This study clearly demonstrated the diversity and bioactive properties of secondary metabolites inhabiting the plant extracts and revealed their antimicrobial and anti-oxidant properties which could have a role in the development of drugs for treatment of a wide spectrum of infectious diseases.

Competing interest: None

Contributions of authors

Ngo Nyobe J.C **conceived and designed the study**; Ngah L,Ngo Nyobe J., Ndom Ntock F, Tse H.P.C, Biwole Bengondo A, Sone B, Ngolsou F**carried out the experiment, analysed and interpreted the results** with help of Ngah L, Ngo Nyobe J.C,. **drafted the manuscript** Ngah L, Ngo Nyobe J.C, Ndom Ntock F, Tse H.P.C, Biwole Bengondo A **revised the manuscript** with help of , Metogo Mbengono J, Fofie Y, Mbosso Teinkela J.E, Etame Loe G, Mpondo Mpondo E **supervised the work at all stages.**

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