

Antimicrobial activity and chemical characterization Fractions Derived from Aqueous and Ethanolic Extracts from *Pteleopsis suberosa* (Combretaceae) and *Acalypha wilkesiana* (Euphorbiaceae)

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Abstract :

Infectious diseases are a global public health issue, exacerbated by the rise of antimicrobial resistance. One possible solution lies in the search for new therapeutic substances in plants. This study aimed to evaluate the antimicrobial activity and chemical characterization of fractions from the extracts of *Pteleopsis suberosa* and *Acalypha wilkesiana* used in traditional Beninese medicine. Aqueous and ethanolic extracts from stem bark of *Pteleopsis suberosa* and the leaves of *Acalypha wilkesiana* were separately fractionated using a liquid-liquid method. Four types of fractions were tested against several pathogens, and their minimum inhibitory concentrations (MIC) were determined. The main phytochemical groups present in these fractions were identified by thin-layer chromatography, flavonoid and total phenol concentrations were measured using spectrophotometric methods. The results revealed that the fractions exhibited varied inhibitory effects, with MIC ranging from 3.12 mg/mL to 100 mg/mL. Most fractions were active on *Candida albicans* ATCC 90028, with the exception of the dichloromethane (DCM) fractions. Only the ethyl acetate (ACE) fraction of the ethanolic extract of *Pteleopsis suberosa* was effective on all tested bacterial strains. The intermediate (INT) fraction of the ethanolic extract of *Pteleopsis suberosa* showed notable efficacy on *Staphylococcus aureus* (MIC = 3.12 mg/mL), while *Escherichia coli* was resistant. Phytochemical analysis identified the presence of tannins, flavonoids, and polyphenols in the fractions, with no significant variation in their concentrations. These compounds may be responsible for their antibacterial and antifungal activities.

Keywords: *Pteleopsis suberosa*, *Acalypha wilkesiana*, Extract fractions, Antimicrobial activity, Benin.

Activité antimicrobienne et caractérisation chimique de fractions d'extraits de *Pteleopsis suberosa* (Combretaceae) et *Acalypha wilkesiana* (Euphorbiaceae)

Résumé :

Les maladies infectieuses constituent un problème de santé publique mondial, aggravé par l'augmentation de la résistance aux antimicrobiens. Une solution possible réside dans la recherche de nouvelles substances thérapeutiques issues des plantes. Cette étude visait à évaluer l'activité antimicrobienne et la caractérisation chimique de fractions provenant d'extraits de *Pteleopsis suberosa* et *Acalypha wilkesiana*, utilisées dans la médecine traditionnelle béninoise. Les extraits aqueux et éthanoliques des écorces de la tige de *Pteleopsis suberosa* et des feuilles de *Acalypha wilkesiana* ont été fractionnés par une méthode liquide-liquide. Quatre types de fractions ont été testés pour leur activité antimicrobienne sur plusieurs pathogènes, et leurs concentrations minimales inhibitrices (CMI) ont été déterminées. Les principaux groupes phytochimiques présents dans ces fractions ont été identifiés par chromatographie sur couche mince, et les teneurs en flavonoïdes et en phénols totaux ont été mesurées par des méthodes spectrophotométriques. Les résultats ont révélé que les fractions présentaient des effets inhibiteurs variés, avec des CMI allant de 3,12 mg/mL à 100 mg/mL. La plupart des fractions étaient actives sur *Candida albicans* ATCC 90028, à l'exception des fractions dichlorométhane (DCM). Seule la fraction éthyl acetate (ACE) de l'extrait éthanolique de *Pteleopsis suberosa* était efficace sur toutes les souches bactériennes testées. La fraction intermédiaire (INT) de l'extrait éthanolique de *Pteleopsis suberosa* a montré une efficacité notable sur *Staphylococcus aureus* (CMI = 3,12 mg/mL), tandis que *Escherichia coli* a été résistante. L'analyse phytochimique a identifié la présence de tanins, flavonoïdes et polyphénols dans les fractions, sans variation significative de leurs concentrations. Ces composés pourraient être responsables de leurs activités antibactériennes et antifongiques.

Mots-clés : *Pteleopsis suberosa*, *Acalypha wilkesiana*, Fractions d'extraits, Activité antimicrobienne, Bénin.

Introduction

Infectious diseases remain a major challenge to global public health. According to recent data, they are responsible for 14 million deaths worldwide and account for 43 % of deaths in developing countries (Antimicrobial Resistance Collaborators, 2022; GBD 2019 Antimicrobial Resistance Collaborators, 2019). In sub-Saharan

Africa, the mortality rate related to infections ranges from 185 to 285 deaths per 100,000 inhabitants (GBD 2019 Antimicrobial Resistance Collaborators, 2019). The mortality rate related to antimicrobial resistance is estimated at 27.3 deaths per year per 100,000 inhabitants (Da et al., 2023). The incidence of infections caused by

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multidrug-resistant strains of antimicrobial agents is a serious public health problem (Prestinaci *et al.*, 2015). This loss of sensitivity in microbial strains, due to either their overuse and misuse (Koudokpon *et al.*, 2021) or the increasing human activities (Omotayo *et al.*, 2017), requires special attention. According to the World Health Organization (WHO) (WHO, 2025) a solution lies in the search for new therapeutic substances in natural resources through a program to fight diseases based on traditional medicine practices (Ikhyameh *et al.*, 2024). Medicinal plants could be a potential source of solutions.

Medicinal plants have long been used in traditional medicine for their various properties, including antimicrobial ones. They have gained increasing interest as an alternative to conventional antimicrobials in recent decades (Sempere-Verdú *et al.*, 2020) due to their many known and proven antimicrobial properties (Bakrim *et al.*, 2022).

Traditional pharmacopeia plants contain a variety of physico-chemical compounds of medical interest, such as polyphenols, terpenes, and alkaloids, which have shown antibacterial properties (Onaebi *et al.*, 2020; Mith *et al.*, 2016; Matasyoh *et al.*, 2008). Today, many phytochemicals are already used as drugs to treat various diseases (Othman *et al.*, 2019) or are considered potential candidates. According to the literature, 60 to 80 % of the pharmaceutical drugs are synthesized from phytochemicals (Castro, 2022; Tsobou *et al.*, 2016; Germanò *et al.*, 1998).

Several ethnopharmacological and pharmacological studies have focused on the antimicrobial properties of *Acalypha wilkesiana* and *Pteleopsis suberosa* (Klotoe *et al.*, 2021; Fanou *et al.*, 2020). They have reported the *in vitro* antimicrobial activity of extracts from these plants (Musa *et al.*, 2024; Klotoe *et al.*, 2021;

Quiroz *et al.*, 2014; Pedersen *et al.*, 2009; Germanò *et al.*, 2008; Baba-Moussa *et al.*, 1999).

The selection of *Pteleopsis suberosa* and *Acalypha wilkesiana* was based on their ethnopharmacological relevance and their documented antimicrobial potential. Both species are widely used in West African traditional medicine for the treatment of infectious diseases, including skin, gastrointestinal, and wound infections. Phytochemical studies have shown that they are rich in tannins, flavonoids, terpenoids, alkaloids, and other phenolic compounds known for their antimicrobial properties (Klotoe *et al.*, 2021, Lagnika *et al.*, 2012). The bacterial and fungal strains investigated in this study were selected because they are among the most common causative agents of human bacterial and fungal infections and are increasingly associated with antimicrobial resistance. Evaluating the activity of plants fractions against these clinically important pathogens may therefore contribute to the identification of promising bioactive compounds for the development of new antimicrobial agents.

However, isolating the pharmacological substances from these plants and using them for therapeutic purposes in the healthcare system remain significant challenges (Santiago *et al.*, 2015; Yoda *et al.*, 2004). Moreover, while studies exploring the antimicrobial activity of *P. suberosa* and *A. wilkesiana* from aqueous, hydroethanolic, and ethanolic extracts (Silén *et al.*, 2023), few have investigated the antimicrobial properties of fractions derived from these extracts.

This study was initiated to evaluate the antimicrobial activity of the fractions from the extracts of *Pteleopsis suberosa* and *Acalypha wilkesiana*. The objective was to identify the most active fraction(s) from these extracts against the tested bacteria and fungi.

1. Material and methods

1.1. Material

1.1.1. Plant material

The plant material consisted of the stem bark of *Pteleopsis suberosa* (AA 6753/ HNB) and the leaves of *Acalypha wilkesiana* (AA 6752/ HNB), collected in Abomey-Calavi's city, (Benin) and authenticated at the National Herbarium of the University of Abomey-Calavi with respectively the numbers AA 6753/ HNB and AA 6752/ HNB.

1.1.2. Microbial strains and reagents

The material used in the study include bacterial strains (*Staphylococcus aureus* ATCC 25925, *Escherichia coli* ATCC 25923, *Pseudomonas*

aeruginosa ATCC 9027, *Klebsiella pneumoniae*) and a fungal strain (*Candida albicans* ATCC 90028). Fluconazole and gentamicin antibiotic discs from Sigma-Aldrich were used as positive controls in the antibacterial activity assays of the extracts.

All chemicals, solvents, culture media, and reference antimicrobial agents used in this study were of analytical or microbiological grade. Sabouraud Dextrose Agar (SDA) and Eosin Methylene Blue agar (EMB) were used for the isolation and cultivation of fungal and bacterial strains, respectively. Mueller-Hinton agar (MHA) was used for antimicrobial susceptibility testing and evaluation of virulence-related

phenotypic characteristics, while Mueller-Hinton broth (MHB) was used for sensitivity assays.

Ethanol was used as the extraction solvent for the preparation of plant extracts. The fractionation process was performed using organic solvents, namely cyclohexane, dichloromethane, and ethyl acetate.

Filtration of extraction mixtures was performed using laboratory filter paper (coffee filter-type paper), funnels were used to facilitate filtration, and glass beakers and flasks were used for preparation and handling of extracts and solvent mixtures. Thin-layer chromatography (TLC) plates and developing chambers were used for fraction separation and chromatographic analysis.

1.2. Methods

1.2.1. Preparation of plant extracts and fractions

Leaves and stem bark from each plant were sorted, dried, and ground into powder to obtain aqueous, hydroethanolic and ethanolic extracts. For the aqueous extracts (Eaq), 100 g of plant powder were boiled in 1 L of distilled water for thirty minutes, followed by triple filtration through absorbent cotton and a final filtration through Whatman No. 2 paper, and then concentrated using a Rotavapor. The hydroethanolic (Eht) and ethanolic (Eth) extracts were obtained by macerating 100 g of powder in 1 L of 96 % ethanol or a hydroalcoholic solution (50% v/v) for 48 hours under magnetic stirring. The homogenates were filtered, concentrated, and dried at room temperature (25 °C) to yield crude plant extracts. The extraction yield (R) of each extract was calculated by dividing the mass of the obtained extract by the mass of the plant powder used (Fanou *et al.*, 2022; Agbodjento *et al.*, 2021).

The extracts that demonstrated antimicrobial activity were fractionated using the liquid-liquid method, adapted from Saptarini and Wardati (2020), and slightly modified by Tokoudagba *et al.* (2022). Briefly, Twenty grams (20 g) of crude extract were dissolved in 50 mL of distilled water and successively partitioned with organic solvents of increasing polarity, namely cyclohexane, dichloromethane, and ethyl acetate. At each partitioning step, 400 mL of the corresponding solvent were added to the aqueous extract in a separatory funnel. The mixture was gently shaken with periodic venting and then allowed to stand for approximately 1 h to ensure complete phase separation. The organic phase was collected, and the extraction was repeated with fresh solvent until the organic

phase became colorless. The remaining aqueous phase was subsequently partitioned with the next solvent in the polarity sequence. The collected fractions were concentrated under reduced pressure using a rotary evaporator, dried at room temperature (25 °C), and stored at 4 °C until use.

1.2.2. Antimicrobial activity of the plant extracts.

The antimicrobial activity of the extracts was assessed using the agar diffusion method on five microbial strains, following the procedure of Fanou *et al.* (2022). The extracts were tested at a concentration of 100 mg/mL and were considered active when the inhibition zone diameter exceeded 6 mm. For antibacterial activity, suspensions of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were prepared in a 0.9 % saline solution at a density of 0.5 McFarland. Five wells were made in Mueller-Hinton (MH) agar plates inoculated with these suspensions: three wells were filled with the extracts, one with gentamicin (10 µg/mL) as a positive control, and one with sterile saline as a negative control. The plates were incubated at 37 °C for 24 hours and then, the inhibition diameters were measured. For antifungal activity, a suspension of *Candida albicans* ATCC 90028 adjusted to a turbidity equivalent to 2 McFarland was used to inoculate Mueller-Hinton agar supplemented with 2 % glucose and 0.5 µg/mL methylene blue (MHA-GMB). Five wells were prepared: three for the extracts, one for fluconazole (100 µg/mL) as a positive control, and one for distilled water as a negative control. After 24 hours of incubation, the inhibition diameters were measured to determine the most active extract. All experiments were performed in triplicate.

1.2.3. Evaluation of the Antimicrobial Activity of Plant Extract Fractions

To evaluate the antimicrobial properties of plant extract fractions, bacterial suspensions (0.5 McFarland) were prepared in a 0.9 % saline solution and inoculated in MH agar plates. Six wells were made in each plate: four wells were filled with 100 µL of each fraction (fr_AQ, fr_ACE, fr_DCM, fr_INT) obtained from the aqueous extract of *Acalypha wilkesiana* (all at 100 mg/mL), while the fifth well was used for gentamicin (10 µg/mL) as a positive control and the sixth, central, well for sterile saline as a negative control (Fanou *et al.*, 2022). After incubating at 37°C for 24 hours, inhibition diameters were measured. The same approach was applied to the ethanolic extract fractions of *Acalypha wilkesiana* and *Pteleopsis suberosa*. For

antifungal activity, a suspension of *Candida albicans* ATCC 90028 (2 McFarland) was used and the fractions were tested similarly, with fluconazole as control. Inhibition diameters were measured to determine the most active fractions (Fanou *et al.*, 2022). All experiments were performed in triplicate.

1.2.4. Determination of MIC and MBC/MFC of extract fractions

The minimum inhibitory concentration (MIC) of the active fractions was determined using the broth microdilution method described by Fanou *et al.* (2022). Briefly, stock solutions of the active fractions were prepared at a concentration of 100 mg/mL. Serial twofold dilutions were performed in sterile broth in 96-well microplates to obtain final concentrations ranging from 100 to 1.5625 mg/mL. Each well received 100 μ L of a standardized microbial suspension, while appropriate positive, negative and reference controls were included. The microplates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration showing no visible microbial growth compared with the growth control. For yeast isolates, absence of growth was confirmed by counting cells using a Malassez haemocytometer under a light microscope ($\times 40$), following the procedure described by Fanou *et al.* (2022). The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined from the MIC assay. Following the incubation period, 10 μ L aliquots were aseptically collected from each well showing no visible microbial growth and subcultured onto Mueller-Hinton agar (MHA) for bacterial strains or Sabouraud Dextrose Agar (SDA) for yeast strains. The inoculated plates were incubated at 37 °C for 24 h. The lowest concentration of the tested fraction that produced no visible colony growth on the agar plates was recorded as the MBC or MFC, indicating complete killing of the bacterial or fungal cells, respectively.

1.2.5. Identification of major chemical groups in fractions

Thin-layer chromatography (TLC) is a technique used for separating and analyzing mixtures of substances, based on the differential migration of components through a thin layer of an absorbent material. In this study, plastic plates coated with a thin layer of silica were used as the stationary phase for the separation of compounds, following the method described by Jazy and Sanogo (2018). The fractions to be analyzed were dissolved in their respective extraction solvents: dichloromethane for fractions obtained with dichloromethane, ethyl acetate for those

extracted with ethyl acetate, and water for the aqueous fraction. The resulting mixtures were applied as small spots on the plate, approximately two centimeters from the lower edge. The plate was then dried and placed in a closed migration chamber containing an eluent system of dichloromethane, ethyl acetate, and water. As the eluent moved through the silica layer by capillary action, it carried the components of the deposited fractions along, resulting in distinct fluorescent or non-fluorescent spots on the plate. Once the solvent front reached the upper edge of the plate, it was removed from the chamber and dried at 100°C. A UV lamp (254 nm and 366 nm) was used to visualize the fluorescent spots, while specific reagents were applied to detect non-fluorescent spots: Dragendorff's reagent for alkaloids, vanillin for phenols, and ferric chloride for tannins.

1.2.6. Determination of polyphenol, flavonoid, and tannin content in fractions

The total polyphenol content in the extracts and active fractions of the studied plants was measured using a modified version of Singleton *et al.* (1999) method, employing the commercial Folin-Ciocalteu reagent. This reagent, a mix of phosphotungstic ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) and phosphomolybdic acids ($\text{H}_3\text{PMO}_{12}\text{O}_{40}$), is reduced during the oxidation of phenols, resulting in a blue color with a maximum absorption at 760 nm, proportional to the polyphenol concentration. A 50 μ L sample of the extract (diluted 1/100 in distilled water) at 25 mg/mL was mixed with 250 μ L of 10% Folin-Ciocalteu reagent and 750 μ L of 75 g/L sodium carbonate solution. After 8 minutes of incubation, 950 μ L of distilled water was added, mixed, and the mixture incubated in darkness at room temperature (25°C) for 2 hours. The optical densities (OD) were then read at 760 nm using a CECIL CE 2041 spectrophotometer, against a blank containing Folin-Ciocalteu reagent, sodium carbonate, and distilled water. The total polyphenol content was determined in mg of gallic acid equivalents per gram of extract (mg GAE/g) using the calibration curve of gallic acid (0-200 μ g/mL) and calculated using the formula of Ahmed *et al.* (2019) :

$$\text{TPT} = (\text{X} \times \text{V})/\text{m}$$

where TPT is the total polyphenol content,
X is the concentration of gallic acid in mg/mL,
V is the volume of extract used in mL,
and m is the mass of the extract in grams.

Each analysis was performed in triplicate. The total flavonoid content in the plant extracts was quantified using a method adapted from

Zhishen *et al.* (1999) and Kim *et al.* (2003), employing aluminum chloride (AlCl_3) as the reagent. AlCl_3 forms a yellow complex with the free hydroxyl groups of flavonoids, and the absorbance of this complex is measured at 415 nm. To perform the assay, 500 μL of a 2% AlCl_3 solution was mixed with 500 μL of the extract and 3 mL of ethanol. The blank consisted of 500 μL of AlCl_3 and 3.5 mL of ethanol. After 10 minutes of incubation, absorbance was measured at 415 nm using a spectrophotometer. The flavonoid content was determined in μg of rutin equivalent per gram of extract ($\mu\text{g RuE/g}$) using a rutin standard curve (0-1 mg/mL). The flavonoid content (TFT) was calculated with the formula:

$$\text{TFT} = (X \times V)/m$$

where X is the rutin concentration in mg/mL,
 V is the volume of extract used in mL,
and m is the mass of the extract in grams.

The tannin content was measured using the vanillin-HCl method Vaillancourt *et al.* (1986), which involves the reaction of vanillin with the flavonoid groups of tannins to form red-colored complexes.

2. RESULTS

2.1. Yields of plant organ extracts.

The yields of the extracts prepared from the plant organs studied are presented in **Erreur ! Source du renvoi introuvable.**

For aqueous extracts (Eaq), the yields were similar for both plants, with 9.2% for *Acalypha wilkesiana* and 9.4% for *Pteleopsis suberosa*,

To quantify condensed tannins, 50 μL of each extract was mixed with 1500 μL of a 4% vanillin/methanol solution and 750 μL of concentrated hydrochloric acid (HCl). The mixture was left at room temperature (25°C) for 20 minutes. Absorbance was then measured at 550 nm against a blank. A calibration curve was generated using catechin standards with concentrations ranging from 0 to 1000 $\mu\text{g/mL}$, yielding the calibration equation :

$$Y = 0.1165X + 0.00003 \text{ with } R^2 = 0.9996.$$

1.2.7. Data Analysis.

The obtained data were subjected to statistical analysis using SPSS 26.0 software and Microsoft Excel 2016. Quantitative variables are presented as means and standard deviations. One-way analysis of variance (ANOVA) was used to assess the variation in the total polyphenol, flavonoid, and tannin content of the studied fractions. When significant differences were detected ($p < 0.05$), multiple comparisons among means were performed using Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was set at $p < 0.05$.

showing no statistically significant difference ($p = 0.757$).

A similar trend was observed for hydroethanolic extracts (Eht).

However, for ethanolic extracts (Eet), the yield was 7.1% for *Acalypha wilkesiana* and significantly higher at 23.63% for *Pteleopsis suberosa* ($p = 0.044$).

Table I: Yields of the prepared extracts

Plant Extracts	Yields (%)		p -values
	<i>Acalypha wilkesiana</i>	<i>Pteleopsis suberosa</i>	
Aqueous (Eaq)	9.20	9.40	0.757
Hydroethanolic (Eht)	16.40	20.56	0.781
Ethanolic (Eet)	7.10	23.63	0.044

2.2. Fractions obtained from the active plant extracts

The fractions obtained from the different active extracts are presented in Table II.

The aqueous, ethyl acetate, and dichloromethane fractions were obtained from the aqueous extracts of *Acalypha wilkesiana* as well as the ethanolic extracts of both plants. However, the intermediate fractions (INT) were only obtained

from the aqueous extract of *Acalypha wilkesiana* and the ethanolic extract of *Pteleopsis suberosa*.

The aqueous extract of *Pteleopsis suberosa* was not subjected to fractionation because it did not exhibit antimicrobial activity during the preliminary screening.

Only extracts with antimicrobial activity were selected for further fractionation.

Table II : Fractions of aqueous and ethanolic extracts of *Acalypha wilkesiana* and *Pteleopsis suberosa*

Types of fractions	E.aq. A.w	Eet. A.w	Eet. P. s
fr_AQ	+	+	+
fr_ACE	+	+	+
fr_DCM	+	+	+
fr_INT	+	-	+

Legend: (+) : Presence; (-) : Absence; **fr:** Fraction ; **AQ:** Aqueous ; **ACE:** Ethyl acetate ; **DCM:** Dichloromethane, **INT:** Intermediate, **Eaq:** Aqueous extract, **A.w:** *Acalypha wilkesiana* ; **Eet :** Ethanolic ; **P.s:** *Pteleopsis suberosa*.

2.3. Antimicrobial properties of active extract fractions from the two studied Plants

The antimicrobial properties of the fractions from the studied plant extracts reveal specific activity profiles. The aqueous extract of *Acalypha wilkesiana* showed no activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, but did exhibit some effectiveness against the growth of *Candida albicans*.

In contrast, the ethanolic extract fractions of *Acalypha wilkesiana* demonstrated enhanced

activity, particularly the DCM fractions, which were notably effective against the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The aqueous fractions (AQ) showed variable activity, including on *Candida albicans*. For *Pteleopsis suberosa*, the ACE fractions of the ethanolic extract were active on all tested strains, and the DCM fraction was highly effective against the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus*, while the INT fractions also showed activity on these bacteria (Table III).

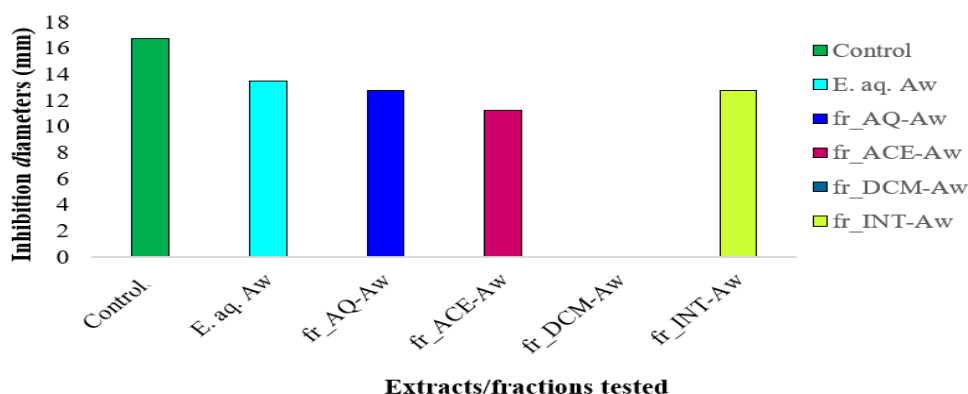
Table III: Inhibition diameters of microorganisms by extracts and fractions of *Acalypha wilkesiana* and *Pteleopsis suberosa*

Extracts/Fractions/Control	Inhibition diameters (mm)				
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Gentamicin (10 µg)/ Fluconazole (100 µg)	23.5 ± 0.71	22	21	24.5 ± 0.71	16.75 ± 0.35
Eaq <i>Acalypha wilkesiana</i>	0	0	0	0	13.5 ± 0.71
Fractions fr_ACE	-	-	-	-	11.25 ± 0.35
fr_DCM	-	-	-	-	0
fr_INT	-	-	-	-	12.75 ± 0.35
fr_AQ	-	-	-	-	12.75 ± 0.35
Eet <i>Acalypha wilkesiana</i>	0	0	10 ± 0.21	13 ± 0.71	9 ± 0.35
fr_ACE	0	0	0	18 ± 1.41	14
Fractions fr_DCM	0	0	20.5 ± 2.12	13.5 ± 2.12	0
fr_AQ	0	0	12.75 ± 0.35	12.75 ± 0.35	11.25 ± 0.35
Eet <i>Pteleopsis suberosa</i>	0	13.25 ± 0.35	10.15 ± 0.21	11	18 ± 1.14
fr_ACE	10.5 ± 0.71	10	9.5 ± 0.71	12.5 ± 3.53	18.5 ± 0.71
Fractions fr_DCM	0	0	0	12.5 ± 2.12	18 ± 1.14
fr_INT	0	11	0	14 ± 2.83	11
fr_AQ	0	0	0	0	18.25 ± 0.35

Legend: E.aq. Aw = Aqueous extract of *Acalypha wilkesiana* ; fr_AQ = Aqueous fraction ; fr_ACE = Ethyl acetate fraction ; fr_DCM = Dichloromethane fraction ; fr_INT = Intermediate fraction; mm = millimeter ; µg = microgram. All assays were performed in triplicate (n = 3) ; values are expressed as mean inhibition diameter ± standard deviation (mm). Values shown without ± indicate a standard deviation of 0 (identical results across the three replicates); "0" indicates no measurable inhibition, (-): not determined within the tested concentration range.

Figure 1 shows the inhibitory effects of the extracts/fractions obtained from *Acalypha wilkesiana* on *Candida albicans* ATCC 90028. The aqueous extract and its fractions aqueous, ethyl acetate, and intermediate effectively inhibited the

growth of *Candida albicans*. However, no inhibitory effect was observed with DCM fraction. Among these, the aqueous fraction of the aqueous extract from *Acalypha wilkesiana* was the most active.



Legend: E.aq. Aw = Aqueous extract of *Acalypha wilkesiana*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction; fr_INT = Intermediate fraction.

Figure 1: Inhibitory effects of extracts/fractions from *Acalypha wilkesiana* on *Candida albicans* ATCC 90028

Legend: *E. coli* = *Escherichia coli*; *K. pneum* = *Klebsiella pneumoniae*; *P. aerug.* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; *C. albicans* = *Candida albicans*; E. eth. Aw = Ethanolic extract of *Acalypha wilkesiana*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction.

Figure 2 illustrates the inhibitory effects of the ethanolic extract and its fractions from *Acalypha wilkesiana* on bacterial strains and *Candida albicans* ATCC 90028. The ethanolic extract and its various fractions were all effective against the growth of *Staphylococcus aureus* ATCC 25925. The *Pseudomonas aeruginosa* ATCC 9027 strain was resistant only to the ACE fraction, while *Candida albicans* ATCC 90028 was resistant only to the DCM fraction. Both *Escherichia coli* and *Klebsiella pneumoniae* strains were resistant to both the extract and its fractions. The aqueous fraction of the ethanolic extract of *Acalypha wilkesiana* displayed the broadest spectrum of activity.

Legend: *E. coli* = *Escherichia coli*; *K. pneum.* = *Klebsiella pneumoniae*; *P. aerug.* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; *C. albicans* = *Candida albicans*; E. eth. Ps = Ethanolic extract of *Pteleopsis suberosa*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction; fr_INT = Intermediate fraction.

Figure 3 displays the inhibitory effects of the ethanolic extract of *Pteleopsis suberosa* and its fractions on bacterial strains and *Candida albicans* ATCC 90028. The results showed that several strains were susceptible to specific fractions. The *Candida albicans* ATCC 90028 strain showed sensitivity to the ethanolic extract and its various

fractions. In contrast, *Staphylococcus aureus* ATCC 25925 was resistant only to the aqueous fraction of this extract. The tested bacilli (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) were most resistant to the ethanolic extract fractions.

The ACE fraction of *P. suberosa* exhibited antibacterial activity against *P. aeruginosa*, with an inhibition zone of 9.5 mm, as shown in Table III. *E. coli* ATCC 25923 was only sensitive to the ACE fraction, while *Klebsiella pneumoniae* was sensitive to both the crude ethanolic extract and the ACE fraction.

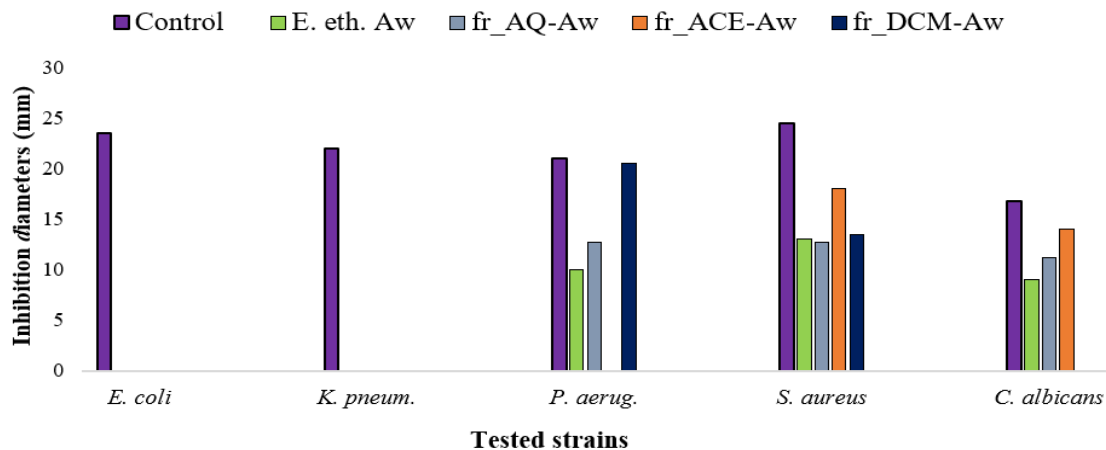
2.4. Minimum Inhibitory Concentrations (MIC) of the studied fractions

The evaluation of minimum inhibitory concentrations (MIC for the active fractions against various microorganisms reveals specific results (Table IV and Table V). For *Candida albicans* ATCC 90028, all four (4) fractions from the ethanolic extract of *Pteleopsis suberosa* (fr_ACE-Eet. Ps, fr_DCM-Eet. Ps, fr_AQ-Eet. Ps, fr_INT-Eet. Ps) exhibited inhibition at concentrations of 50 mg/mL, 50 mg/mL, 50 mg/mL, and 25 mg/mL, respectively. The aqueous and intermediate fractions of the aqueous extract of *A. wilkesiana* showed equal antifungal activity against *C. albicans*, both producing an inhibition zone of 12.75 mm.

Regarding *Escherichia coli* ATCC 25923, the MIC for the ACE fraction of the ethanolic extract of *Pteleopsis suberosa* was 100 mg/mL. For *Pseudomonas aeruginosa* ATCC 9027, the MIC for the DCM and aqueous fractions of the ethanolic

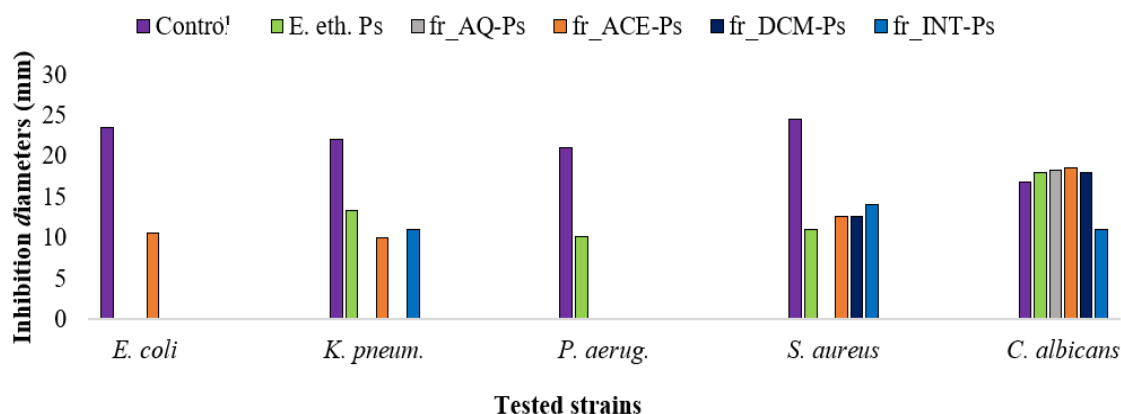
extract of *Acalypha wilkesiana* were both 100 mg/mL, while the MIC for the ACE fraction of the ethanolic extract of *Pteleopsis suberosa* was 50 mg/mL. For *Staphylococcus aureus* ATCC 25925, the MIC for the ACE, DCM, and aqueous fractions of the ethanolic extract of *Acalypha*

wilkesiana, as well as the ACE fraction of the ethanolic extract of *Pteleopsis suberosa*, were all 100 mg/mL. In contrast, the INT and DCM fractions of the ethanolic extract of *Pteleopsis suberosa* had MIC values of 3.12 mg/mL and 25 mg/mL, respectively (Table I).



Legend: *E. coli* = *Escherichia coli*; *K. pneum.* = *Klebsiella pneumoniae*; *P. aerug.* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; *C. albicans* = *Candida albicans*; E. eth. Aw = Ethanolic extract of *Acalypha wilkesiana*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction.

Figure 2: Inhibitory effects of the ethanolic extract and its fractions from *Acalypha wilkesiana* on bacterial strains and *Candida albicans* ATCC 90028



Legend: *E. coli* = *Escherichia coli*; *K. pneum.* = *Klebsiella pneumoniae*; *P. aerug.* = *Pseudomonas aeruginosa*; *S. aureus* = *Staphylococcus aureus*; *C. albicans* = *Candida albicans*; E. eth. Ps = Ethanolic extract of *Pteleopsis suberosa*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction; fr_INT = Intermediate fraction.

Figure 3: Inhibitory effects of the ethanolic extract and its fractions from *Pteleopsis suberosa* on bacterial strains and *Candida albicans* ATCC 90028.

Table IV: Minimum Inhibitory Concentrations of active fractions against *Candida albicans* ATCC 90028, *Escherichia coli* ATCC 25923, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25925

Strains	Fraction	Dilution							MIC (mg/mL)	MBC (mg/mL)
		N	1/2	1/4	1/8	1/16	1/32	1/64		
<i>Candida albicans</i> ATCC 90028	fr_ACE-Eaq. Aw	+	-	-	-	-	-	-	100	-
	fr_INT-Eaq. Aw	+	-	-	-	-	-	-	100	-
	fr_AQ-Eaq. Aw	+	+	+	+	-	-	-	12.5	-
	fr_ACE-Eet. Aw	+	-	-	-	-	-	-	100	-

	fr_ACE-Eet. Ps	+	+	-	-	-	-	-	50	-
	fr_DCM-Eet. Ps	+	+	-	-	-	-	-	50	-
	fr_AQ-Eet. Ps	+	+	-	-	-	-	-	50	-
	fr_INT-Eet. Ps	+	+	-	-	-	-	-	25	-
<i>Escherichia coli</i> ATCC 25923	fr_ACE-Eet. Ps	+	-	-	-	-	-	-	100	-
	fr_ACE-Eet. Ps	+	-	-	-	-	-	-	100	-
<i>Klebsiella pneumoniae</i>	fr_INT-Eet. Ps	+	+	+	-	-	-	-	25	-
<i>P. aeruginosa</i> ATCC 9027	fr_AQ-Eet. Aw	+	-	-	-	-	-	-	100	-
	fr_DCM-Eet. Aw	+	-	-	-	-	-	-	100	-
	fr_ACE-Eet. Ps	+	+	-	-	-	-	-	50	-

Legend: N = Initial concentration of 100 mg/mL; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; (+) = inhibition; (-) = microbial growth or regrowth (in the dilution series); in the MBC/MFC column, (-) indicates that the bactericidal/fungicidal concentration was not reached within the tested concentration range (i.e., MBC/MFC > 100 mg/mL). E.aq. Aw = Aqueous extract of *Acalypha wilkesiana*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction; fr_INT = Intermediate fraction.

Table I: Minimum Inhibitory Concentrations (MIC) of active fractions against *Candida albicans* ATCC 90028, *Escherichia coli* ATCC 25923, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25925

Microorganism Strains		N	Dilutions						Concentrations (mg/mL)	
			1/2	1/4	1/8	1/16	1/32	1/64	MIC	MBC
<i>Staphylococcus aureus</i> ATCC 25925	fr_ACE- Eet. <i>Aw</i>	+	-	-	-	-	-	-	100	-
	fr_AQ- Eet. <i>Aw</i>	+	-	-	-	-	-	-	100	-
	fr_DCM- Eet. <i>Aw</i>	+	-	-	-	-	-	-	100	-
	fr_ACE- Eet. <i>Ps</i>	+	-	-	-	-	-	-	100	-
	fr_DCM- Eet. <i>Ps</i>	+	+	+	-	-	-	-	25	-
	fr_INT- Eet. <i>Ps</i>	+	+	+	+	+	+	-	3.12	-

Legend: N = Initial concentration of 100 mg/mL; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; (+) = inhibition; (-) = microbial growth or regrowth (in the dilution series); in the MBC/MFC column, (-) indicates that the bactericidal/fungicidal concentration was not reached within the tested concentration range (i.e., MBC/MFC > 100 mg/mL). E.aq. Aw = Aqueous extract of *Acalypha wilkesiana*; fr_AQ = Aqueous fraction; fr_ACE = Ethyl acetate fraction; fr_DCM = Dichloromethane fraction; fr_INT = Intermediate fraction

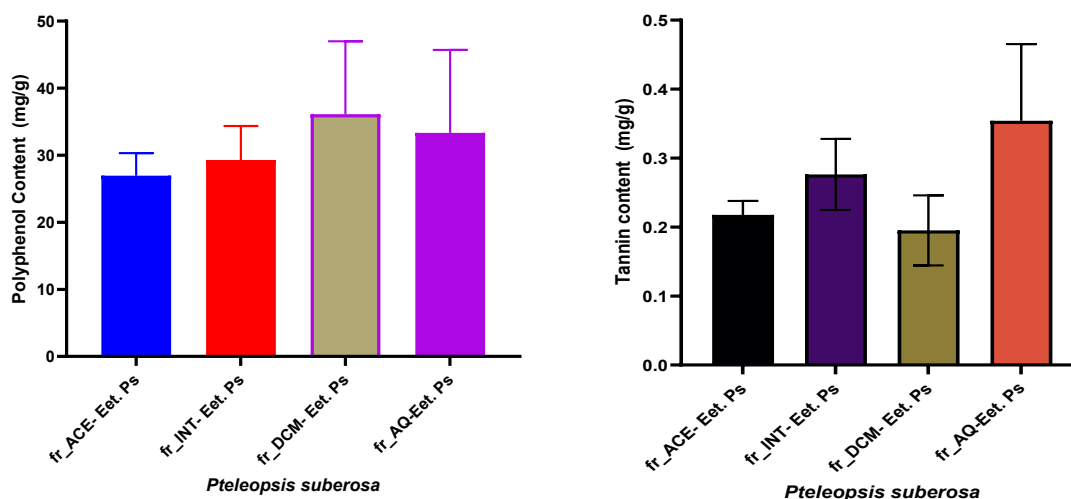
Three phenolic parameters were quantified in the ethanolic extracts of *Acalypha wilkesiana* and *Pteleopsis suberosa*, as well as in their various fractions: total polyphenol content, together with two major polyphenol subclasses, flavonoids and tannins.

2.5. Polyphenol, Flavonoid, and Tannin content of *Pteleopsis suberosa*

The assessment of polyphenol, flavonoid, and tannin content in the different fractions of *Pteleopsis suberosa* showed that fr_DCM- Eet. Ps had the highest average concentration of polyphenols, followed by fr_AQ-Eet. Ps, fr_INT-Eet. Ps, and fr_ACE-Eet. Ps of *Pteleopsis suberosa* (Figure 4a). Despite this, statistical analysis

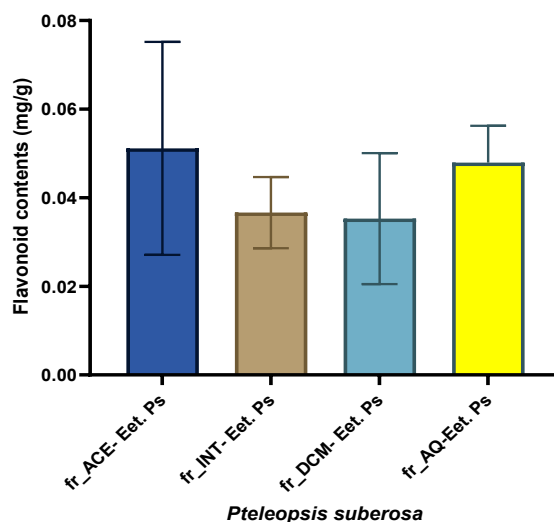
revealed no significant differences in the concentrations of polyphenols, flavonoids, and tannins among these fractions ($p > 0.05$).

The flavonoid content was lower in the fr_INT-Eet. Ps and fr_DCM- Eet. Ps fractions compared to the fr_ACE-Eet. Ps fraction, but again, there were no significant differences ($p > 0.05$) (Figure 4b). Additionally, the fr_AQ-Eet. Ps had the highest concentration of tannins, followed by fr_INT-Eet. Ps and fr_ACE-Eet. Ps. Statistical analysis also indicated no significant differences in tannin content across the various fractions ($p > 0.05$) (Figure 4c).



a) Polyphenol content of the various fractions of the ethanolic extract of *Pteleopsis suberosa*

c) Tannin content in the different fractions of the ethanolic extract of *Pteleopsis suberosa*



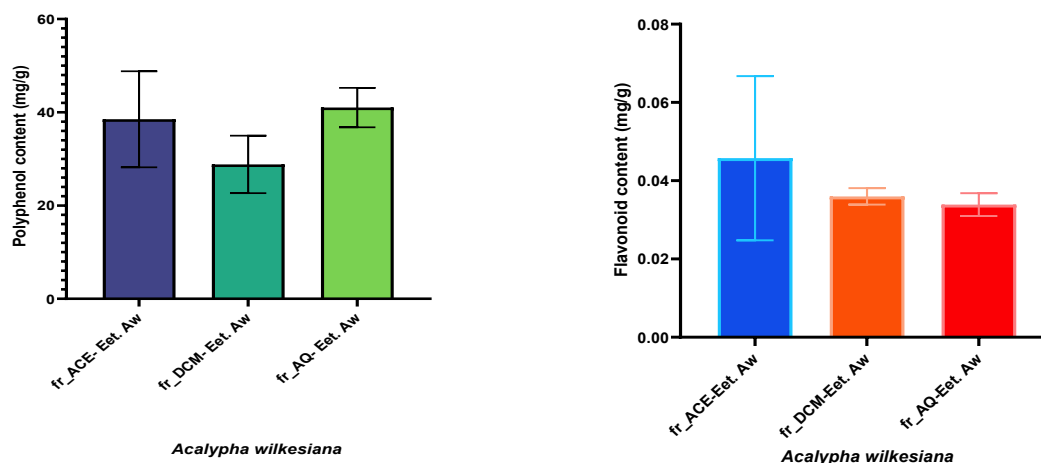
b) Flavonoid content in the different fractions of the ethanolic extract of *Pteleopsis suberosa*

Figure 4: Polyphenol, Flavonoid, and Tannin content in the different fractions of the ethanolic extract of *Pteleopsis suberosa*;

2.6. Polyphenol, Flavonoid, and Tannin Content in *Acalypha wilkesiana*

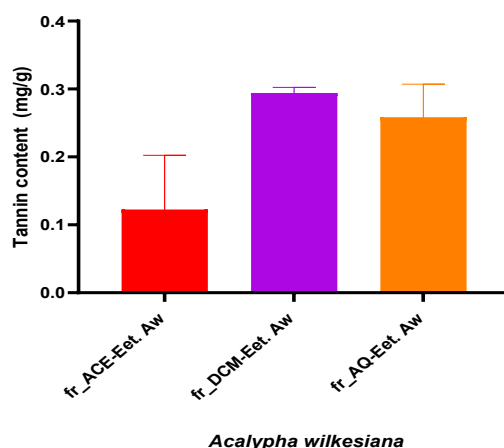
The analysis of polyphenol, flavonoid, and tannin content in the different fractions of *Acalypha wilkesiana* revealed variability among the fractions. The fr_AQ-Eet. Aw had the highest average concentration of polyphenols, followed by the fr_ACE-Eet. Aw fraction (Figure 5a).

The fr_ACE-Eet. Aw fraction was richest in flavonoids (Figure 5b). The fr_DCM-Eet. Aw fraction had the highest concentration of tannins and the fr_ACE-Eet. Aw fraction had the lowest (Figure 5c). Statistical analysis revealed no significant differences in polyphenol, flavonoid, or tannin content across the various fractions of *Acalypha wilkesiana* ($p > 0.05$).



a) Polyphenol content of the various fractions of the ethanolic extract of *Acalypha wilkesiana*

b) Flavonoid content of the various fractions of the ethanolic extract of *Acalypha wilkesiana*



c) Tannin content of the various fractions of the ethanolic extract of *Acalypha wilkesiana*

Figure 5: Polyphenol, Flavonoid, and Tannin Content in *Acalypha wilkesiana*

3. Discussion

The aim of this study was to evaluate the antimicrobial activity and chemical characterization of extract fractions from *Pteleopsis suberosa* and *Acalypha wilkesiana*.

The extraction yields varied from 7.1 % à 23. 63 % depending on the plants and solvents used. Specifically, the ethanolic extracts of *Acalypha wilkesiana* leaves and *Pteleopsis suberosa* bark yielded 7.10 % and 23.63 %, respectively. High yields were mainly observed for ethanolic extracts of the plants.

These results are lower than those reported by Fanou *et al.* (2022), who obtained yields of 26 % for *Acalypha wilkesiana* and 31.13 % for *Pteleopsis suberosa*. Additionally, unlike our results, Fanou *et al.* (2022) achieved the best yields with aqueous

extracts of the studied plants. Literature, as reported by Olawuwo *et al.* (2022), also indicates high yields for aqueous extracts of *Acalypha wilkesiana* and other plant species. This difference in yields may be attributed to various factors, including the timing of the harvest of the plant parts used.

In addition to the harvesting period, extraction yields are strongly influenced by several factors, including the geographical origin of the plant material, climatic and edaphic conditions, the physiological stage of the plant, drying conditions, particle size, solvent polarity (Nausheen Ghaffar & Anjum Perveen, 2024), extraction time, and extraction temperature. These factors may alter both the quantity and the

composition of extractable secondary metabolites, thereby explaining the variability observed among studies.

Only the aqueous extract of *Acalypha wilkesiana* and the ethanolic extracts of both plants could be fractionated given their performances. The aqueous extract of *Pteleopsis suberosa* could not be fractionated, likely due to the method used, according to Abubakar and Haque (2020).

The obtained fractions showed varied antimicrobial effects on the tested strains. All fractions of the ethanolic extracts of *Acalypha wilkesiana* and *Pteleopsis suberosa*, except for the DCM fraction of the ethanolic extract of *Acalypha wilkesiana*, exhibited antifungal activity against *Candida albicans* growth. These results are consistent with reports on the ability of extracts from *Acalypha wilkesiana* and *Pteleopsis suberosa* to inhibit the growth of microscopic fungi (Silén et al., 2023; Ble-Gonzalez et al., 2022; Klotoe et al., 2021 ; Singh and Fardami, 2018). Fabunmi et al. (2016) also demonstrated that the crude ethyl acetate extract of *Acalypha wilkesiana* was active against *Candida albicans* growth. Moreover, Lagnika et al. (2012) showed that extracts obtained from dichloromethane, methanol, and ethanol inhibited fungal strains.

Differences in antifungal activity among studies may result from variations in extraction procedures, solvent polarity, fractionation strategies, fungal strains tested, inoculum density, and susceptibility testing protocols. Furthermore, fractionation may separate bioactive compounds that act synergistically in crude extracts, resulting in reduced activity of individual fractions.

Regarding antibacterial activity, the obtained fractions had variable effects. Several studies did not use fractions derived from plant extracts but rather fractions prepared directly from solvents (Musa et al., 2024; Lagnika et al., 2012; Vaillancourt et al., 1986). Fabunmi et al. (2016) reported a MIC of 40 mg/mL for *Acalypha wilkesiana* on *Candida albicans*, compared to 100 mg/mL in our study. This variability in inhibitory effects could be due to incomplete extraction of active compounds in the tested fractions compared to those obtained directly from solvents.

Another possible explanation is that crude extracts often contain complex mixtures of phytochemicals that exert synergistic antibacterial effects. During fractionation, these synergistic interactions may be partially lost as compounds are separated into different fractions,

leading to higher MIC values than those observed for crude extracts.

Inhibition of the tested bacteria was observed for most fractions only at a concentration of 100 mg/mL. In contrast to our results, Ble-González et al. (2022) achieved MIC greater than 2 and equal to 5 mg/mL for the crude ethyl acetate extract of *Acalypha wilkesiana* against the tested strains, and Fabunmi et al. (2016). found similar results on *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*. The inhibitory effects of the compounds may be dose-dependent. Residues from aqueous or ethanolic extracts from which our fractions were obtained may contain active compounds that were not fully extracted. Among all the obtained fractions, the ethyl acetate fraction of *Pteleopsis suberosa* was the only one active against all tested microorganism strains. These results are in agreement with those obtained by Ngounou et al. (1999) on *Pteleopsis hyloidendron*, a species from the same genus as *Pteleopsis suberosa*. These authors showed that the ethyl acetate fraction of this plant inhibited strains such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which were also tested in the present study. Ethyl acetate is considered a semi-polar solvent capable of concentrating medium-polarity phenolic compounds, particularly ellagitannins and flavonoids, which have been associated with antimicrobial activity in other *Acalypha* species (Ble-Gonzalez et al., 2022). As these individual compounds were not isolated or identified in the present study, this association remains a plausible hypothesis; the higher activity of this fraction nonetheless suggests that such compounds may contribute to the observed biological effects.

The fractions were more active against *Staphylococcus aureus* strains than against other strains, while *E. coli* proved to be more resistant. Our results corroborate those of Fabunmi et al. (2016) but diverge regarding the resistance of *Pseudomonas aeruginosa*. Olawuwo et al. (2022) showed that *Escherichia coli* strains were more sensitive than the staphylococci strains tested. The greater susceptibility of Gram-positive bacteria compared with Gram-negative bacteria has been widely reported. This difference is mainly attributed to the structural organization of the bacterial cell envelope. Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides, which limits the penetration of many antimicrobial compounds, particularly polyphenols and tannins. In contrast, Gram-positive bacteria lack this outer membrane,

making them generally more susceptible to plant-derived antimicrobial agents.

Phenolic compounds, together with two of their major subclasses, flavonoids and tannins, were quantified in both raw extracts and their fractions. The antimicrobial activity of these phytochemicals has been extensively documented. Flavonoids may inhibit nucleic acid synthesis, disrupt cytoplasmic membranes, and interfere with energy metabolism. Hydrolysable tannins, including ellagitannins such as corilagin, can precipitate microbial proteins, inhibit extracellular enzymes, and alter membrane permeability. Polyphenols also induce oxidative stress and damage microbial cell structures. The combined action of these compounds may explain the antimicrobial activity observed in the present study. The presence of tannins in the studied fractions is confirmed by several recent studies using various methods (Ble-Gonzalez *et al.*, 2022; Osibote *et al.*, 2021; Adejoke *et al.*, 2020; Zubairu *et al.*, 2020; Mustafa 2014). Ellagitannins, particularly corilagin, are the main compounds in these tannins. These studies also reported the presence of polyphenols and flavonoids in the fractions of *Acalypha wilkesiana*.

Regarding the compounds of *Pteleopsis suberosa*, our results are consistent with recent studies (Pascal *et al.*, 2023; Fanou *et al.*, 2021) but differ from those of Lagnika *et al.* (2012) regarding the presence of flavonoids. These discrepancies may be related to differences in extraction solvents, phytochemical screening methods, geographical origin of the plant material, seasonal variation, or genetic variability within the species.

The antibacterial and antifungal activities of the studied fractions would be related to the presence of phenolic compounds, particularly flavonoids and tannins, identified in variable amounts but without significant differences (Lagnika *et al.*, 2012; Germanò *et al.*, 2008).

Overall, the present findings confirm that fractionation influences the antimicrobial activity of plant extracts by modifying the distribution of bioactive compounds. Although some fractions exhibited lower antimicrobial activity than crude extracts, the ethyl acetate fraction of *Pteleopsis*

suberosa retained broad-spectrum activity, suggesting that this fraction is enriched in compounds with promising antimicrobial potential. Further purification and structural characterization of these compounds are warranted.

A limitation of our study is the lack of investigation on saponins, alkaloids, anthraquinones, and anthocyanins, which are also known for their antimicrobial properties (Yoda *et al.*, 2004; Ngounou *et al.*, 1999).

Another important limitation is that the present study did not evaluate the toxicity or safety profile of the active extracts and fractions. Consequently, although the observed antimicrobial activities are promising, these findings should be considered preliminary. Additional toxicological studies, including cytotoxicity assays and *in vivo* safety evaluations, are required before these fractions can be considered as potential candidates for phytomedicine development.

Building on these findings, future work should prioritize three complementary directions. First, extraction and fractionation conditions should be optimized, particularly for the aqueous extract of *Pteleopsis suberosa*, whose extraction yield remained low, by systematically varying parameters such as temperature, duration, and solvent polarity, and by testing alternative fractionation techniques to obtain more representative, higher-yield fractions. Second, the active fractions should be evaluated against a broader panel of pathogenic strains, and their bioactive constituents should be identified and characterized using high-performance liquid chromatography (HPLC) and mass spectrometry (MS), which would help pinpoint the specific compounds responsible for the observed antimicrobial activity.

Third, comprehensive *in vitro* and *in vivo* toxicological and pharmacological studies, including assessments of possible synergistic interactions among constituents, will be essential to establish the safety and therapeutic potential of these fractions before any consideration of medicinal application.

Conclusion

This study provided the important data on the antimicrobial activities and chemical characterization of active extracts from *Acalypha wilkesiana* and *Pteleopsis suberosa*. Fractions of ACE, INT, AQ, and DCM were obtained from the aqueous and ethanolic extracts of both plants, with the exception of the aqueous extract of

Pteleopsis suberosa, for which no fractions were identified.

These fractions exhibited varying inhibitory capacities against bacterial and fungal growth, with minimum inhibitory concentrations (MIC) ranging from 3.12 mg/mL to 100 mg/mL. Among them, the ACE fraction of the ethanolic

extract of *Pteleopsis suberosa* exhibited the broadest antimicrobial spectrum, being the only fraction active against all five tested microorganisms, and *Staphylococcus aureus* ATCC 25925 was the most sensitive strain to the different fractions.

Chemical analyses revealed that all fractions contained varying concentrations of tannins, flavonoids, and polyphenols. The observed antimicrobial properties are likely related to the richness of these fractions in tannins and other

phenolic compounds. Consequently, the bark of *Pteleopsis suberosa* and the leaves of *Acalypha wilkesiana* represent promising sources of bioactive compounds with antimicrobial potential against the tested microorganisms. However, further studies, including comprehensive toxicological evaluations and *in vivo* pharmacological investigations, are required to confirm their safety, efficacy, and therapeutic potential before considering any medicinal application.

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