

In vitro Antioxidant, Antimicrobial and Bioassay Guided-Fractionation of Methanol Extract of *Pericopsis Laxiflora* Leaf

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Abstract- This study investigated the *in vitro* antioxidant and antimicrobial activities of the Methanol extract of *Pericopsis laxiflora* leaf (MLPL) and its bioassay-guided fractions, aiming to identify the most active constituents. Phytochemical screening of MLPL and its fractions revealed the presence of flavonoids, anthraquinones, tannins, saponins, and alkaloids, with notable variations across fractions. Antioxidant activity was evaluated using DPPH radical scavenging, lipid peroxidation (MDA), nitric oxide, superoxide radical scavenging, and reducing power assays. The ethyl acetate (EAFPL) and butanol (BFPL) fractions consistently demonstrated superior antioxidant activity, with EAFPL showing the highest potency across all models, correlating with its high phenolic content. Antimicrobial activity was assessed against bacterial pathogens (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*) and fungal strains (*Candida albicans*, *Aspergillus spp.*). EAFPL again exhibited the strongest inhibition, indicating broad-spectrum antimicrobial potential. The study highlights the relevance of bioassay-guided fractionation in isolating potent phytochemicals responsible for biological activity. Clinically, the antioxidant and antimicrobial properties of EAFPL suggest potential therapeutic applications in oxidative stress-related diseases and microbial infections. Overall, *P. laxiflora* leaves are a promising source of bioactive compounds which serve as intervention in the management of oxidative stress-linked diseases and microbial infections.

Index Terms - Antimicrobial, Bio-Guided Fractionation, Medicinal Plants, Oxidative Stress, Phytochemical Profile.

I. INTRODUCTION

Oxidative stress describes a biological state in which the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceeds the capacity of endogenous antioxidant defenses, resulting in

damage to lipids, proteins, and nucleic acids and disruption of redox-sensitive signaling pathways (Chaudhary et al., 2023; Krishnamurthy et al., 2024; Qin et al., 2026). Although ROS/RNS at physiological levels contribute to essential functions such as immune defense and cellular signaling, persistent oxidative imbalance is strongly implicated in the initiation and progression of multiple acute and chronic disorders, including inflammatory conditions, metabolic disease, neurodegeneration, cardiovascular dysfunction, and cancers (Chaudhary et al., 2023; Qin et al., 2026). Because oxidative injury often involves lipid peroxidation and related biomarker changes, antioxidant discovery and evaluation continues to be a priority for preventing or mitigating oxidative stress-linked pathologies (Krishnamurthy et al., 2024).

In parallel, infectious diseases remain a major driver of morbidity and mortality worldwide, and treatment is increasingly complicated by antimicrobial resistance (AMR). Large-scale global analyses show that bacterial AMR is a leading cause of death, particularly in low-resource settings, with high-burden pathogen-drug combinations including resistant *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Murray et al., 2022). The World Health Organization has emphasized AMR as a top global health and development threat, and the updated WHO Bacterial Priority Pathogens List (2024) highlights resistant bacterial pathogens requiring urgent research attention, including *S. aureus*, *P. aeruginosa*, and *Salmonella* among others (World Health Organization [WHO], 2023, 2024). Recent forecasting work further underscores the urgency by quantifying long-term trends and projecting

substantial future AMR burden if effective countermeasures are not accelerated (GBD 2021 Antimicrobial Resistance Collaborators, 2024).

Natural products research, especially from medicinal plants, continues to contribute promising chemical diversity for both antioxidant and antimicrobial lead discovery. Plant secondary metabolites, notably polyphenols (including flavonoids and related phenolics), can act as redox-active compounds that neutralize radicals or interrupt radical-driven chain reactions, while also exhibiting antimicrobial effects through membrane disruption, interference with microbial enzymes, or perturbation of cellular homeostasis (De Rossi et al., 2025; Vaou et al., 2021). Contemporary evidence also supports the concept that complex phytochemical mixtures may provide multi-target effects and, in some cases, augment antimicrobial efficacy when compared across extraction methods and phytochemical profiles (Zouine et al., 2024). This dual relevance is particularly important in disease contexts where oxidative stress and infection/inflammation co-exist, making plants with combined antioxidant and antimicrobial activities attractive candidates for further study (De Rossi et al., 2025).

A recurring challenge in botanical drug discovery is that crude extracts are chemically complex and may contain hundreds of metabolites with overlapping, synergistic, or antagonistic activities. Bioassay-guided fractionation addresses this limitation by sequentially separating extracts into fractions and repeatedly testing them to localize biological activity and enrich for active constituents, thereby providing a rational pathway toward isolation and characterization of bioactive compounds (Mani et al., 2022; Jović et al., 2023). Modern workflows frequently integrate solvent–solvent partitioning, chromatographic separation, and targeted assays to accelerate identification of active zones or fractions before deeper structural elucidation (Jović et al., 2023). Recent open-access examples continue to demonstrate the utility of bioassay-guided strategies for discovering antimicrobial or multi-bioactive constituents from plant materials (Baldé et al., 2026; Mani et al., 2022).

Pericopsis laxiflora (Fabaceae) is a West African medicinal plant with longstanding ethnomedicinal use and expanding pharmacological interest. A recent focused review synthesizing the plant's ethnobotany, phytochemistry, and pharmacology reports its traditional use for conditions including gastrointestinal disorders, fever, pain-related complaints, and skin diseases, and highlights published evidence for antimicrobial and antioxidant activities (Sarfo-Antwi et al., 2021). Safety profiling has also been reported, including an in vivo acute and sub-acute toxicity assessment of an aqueous preparation (Ouattara et al., 2020). Importantly, contemporary studies continue to broaden the evidence base: volatile oils from leaf and stem have been shown to possess antioxidant activity (DPPH-based) and antimicrobial effects against a panel of bacterial and fungal organisms (Oloyede & Bello, 2022), while stem bark preparations have been investigated for fraction-dependent protection in oxidative stress–linked injury models (Sarfo-Antwi et al., 2024) and for toxicity/anti-inflammatory outcomes relevant to malaria-associated inflammation (Jeanne et al., 2026). Together, these findings support *P. laxiflora* as an active reservoir of bioactive metabolites, while also indicating that activity may vary substantially by plant part, extraction solvent, and fractionation strategy (Oloyede & Bello, 2022; Sarfo-Antwi et al., 2024).

Despite this progress, there remains a practical need, aligned with both pharmacological relevance and product development goals, to identify which *P. laxiflora* leaf fractions concentrate the most potent antioxidant and antimicrobial constituents under standardized in vitro conditions. Therefore, the present study evaluated the methanol extract of *P. laxiflora* leaves and its bioassay-guided solvent fractions, profiling phytochemical classes and assessing antioxidant activity across multiple complementary models (DPPH radical scavenging, nitric oxide and superoxide scavenging, reducing power, and lipid peroxidation inhibition) alongside antimicrobial screening against clinically relevant bacterial and fungal pathogens.

The overarching objective was to localize activity to the most effective fraction(s), providing a justified starting point for future compound-level

identification and development in the context of oxidative stress-related conditions and AMR-era infectious disease challenges.

II. MATERIALS

In this study, every chemical utilized were of Analar grade. Catechin, Sodium Nitrite, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Griess reagent, Nitro blue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB), butylated hydroxytoluene (BHT), potato dextrose agar (PDA) and Nutrient agar were procured from Sigma Aldrich Chemical Company through their sales agents in Nigera. Thiobarbituric acid (TBA), Sodium salt of ethylenediamine tetraacetic acid (EDTA-Na), saline phosphate buffer (SPB), ascorbic acid, dimethyl sulphoxide (DMSO), trichloroacetic acid (TCA), ammonium sulphate, ferric chloride (FeCl₃), dihydrate sodium nitroprusside (Na₂[Fe(CN)₅NO]·2H₂O), cadmium chloride, acetic acid, and potassium ferricyanide were of analytical grade and were gotten from Seglor Nigeria limited. 752N UV-visible spectrophotometer was employed for all spectroscopic experiments.

Plant Material collection and preparation

The leaves of *Periscopsis laxiflora* were freshly collected from the garden of the Cocoa Research Institute of Nigeria, Ibadan, Oyo State. The leaves were dehydrated at room temperature, blended and extracted in methanol by cold maceration where 5000 mg of the dried blended leaves were drenched in 1.4L of 85% methanol for 72 hours at 25°C. The mixture was filtered and thereafter concentrated in a rotary evaporator and further dried to a constant weight. The crude extract was then kept at 20°C for further study.

Bioassay guided- Fractionation

The crude extract of *Periscopsis laxiflora* was subjected to bio-guided fractionation via solubilization in water and subsequent sequential partition with hexane in 5 volumes, Butanol (4 Volumes), ethyl acetate (5 volumes), chloroform (3 Volumes), ethanol (5 Volumes) and 50% methanol (3 Volumes) as indicated in Figure 1. Each fraction thus obtained, including the final hydro-methanol fraction, was evaporated to dryness in a rotary evaporator and

subjected to phytochemical analysis, *in vitro* antioxidant assay and Antimicrobial analysis.

Phytochemical Analysis

The crude methanol extract of the leaf of *P. laxiflora* was screened for its phytochemical constituents using standard procedures following Harborne and Baxter's (1993) methods to detect secondary metabolites. The methods used for Alkaloids, Flavonoids, Tannins, cardiac Glycosides, Saponins and Anthraquinones were Dragendorff's and Wagner's tests, Shinoda test, Ferric chloride test, Keller-Killani test, Frothing test and Bornträger's test respectively.

In-vitro antioxidant activities

DPPH radical scavenging activity: The free radical scavenging ability of the crude and fractions of *P. laxiflora* against DPPH free radical was evaluated by the method described by Oyediji *et al.* (2025).

Nitric oxide (NO) and Superoxide radical scavenging activity: Nitric oxide (NO) radical scavenging activity of the crude and fractions of *P. laxiflora* were determined according to the method of Tijani *et al.* (2021).

Lipid peroxidation inhibition: Lipid peroxidation, measured as the dose-dependent inhibition of Malonaldehyde, of the crude and fractions of *P. laxiflora* was evaluated by a modified method of Anifowose *et al.* (2025).

Ferric (Iron II) reducing potential: This assay was used to test the potential of the crude and fractions of *P. laxiflora* to bring about the reduction of iron II to iron III and was evaluated following the method of Sanda *et al.* (2021) where 2 mL of the sample was transferred into 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. This was followed by incubation at 50°C for 20 min., cooled and then 2.5 mL of Trichloroacetic Acid (10%) was introduced into the mixture and was centrifuged at 5000 rpm for 10 minutes. Afterwards, 2.5 mL of the supernatant was transferred into test tube containing 2.5 mL of distilled water while 0.5 mL of ferric chloride (0.1%) was added and was then kept on the bench for 10 minutes and the absorbance was taken at 700 nm

Antimicrobial Activity

Preparation of microorganisms: The bacteria and fungi isolate used in this study was obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State which includes the Gram-positive ones (*Streptococcus pneumonia* and *Staphylococcus aureus*) Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and fungi (*Aspergillus niger* and *Candida albicans*). The selection of these organisms was based on their pharmacological and clinical importance.

Antimicrobial susceptibility testing: The sensitivity of microorganisms against the crude and fractions of *P. laxiflora* was elucidated using agar well diffusion method as outlined by the International Commission on Microbiological Specifications for Foods (ICMSF, 1998) where the degree of inhibition was measured as the diameter of the inhibition zone while standard antibacterial – Augmentin and Antifungi – Griseofulvin were used reference.

III. RESULTS

Table 1: Phytochemical constituents of Crude and Fractions of *P. laxiflora* Leaf extracts.

Phytochemicals	MLPL	HFPL	BFPL	EAFPL	CFPL	HEFHB
Anthraquinone	++	--	++	++	--	--
Flavonoids	++	--	++	++	--	++
Alkaloids	++	++	--	++	--	++
Saponins	++	++	--	--	++	--
Phlobatannis	++	--	++	--	++	--
Cardiac glycosides	--	--	--	--	—	--
Tannis	--	--	--	++	++	--

MLPL- Methanol leaf extract of *Periscopsis laxiflora*, HFPL- Hexane fraction of Methanol leaf extract of *Periscopsis laxiflora*, BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*, CFPL- Chloroform fractoin of Methanol leaf extract of *Periscopsis laxiflora*, EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*, HEFPL- Hydro-Ethanol fraction of Methanol leaf extract of *Periscopsis laxiflora*; ++ = present, -- = absent

Table 2: Inhibitory activity of the Crude and Fractions of Methanol extract of *Pericopsis laxiflora* leaf (MLPL) against DPPH radicals relative to Catechin

CONC. (mg/mL)	% INHIBITION OF DPPH					
	MLPL	HFPL	BFPL	EAFPL	CFPL	CATECHIN
Control	0.000	0.000	0.0000	0.000	0.000	0.000
100	21.45 ± 1.08 ^{ab}	-17.43 ± 3.16 ^{ab}	32.32 ± 7.54 ^b	09.34 ± 7.26 ^{ab}	-3.72 ± 0.32 ^{ab}	35.24 ± 3.11 ^b
200	39.82 ± 3.52 ^b	-25.53 ± 3.89 ^{ab}	38.95 ± 5.39 ^{ab}	24.56 ± 8.23 ^{ab}	7.92 ± 1.03 ^a	44.37 ± 6.23 ^b
500	43.74 ± 4.02 ^{ab}	13.16 ± 3.24 ^{ab}	47.02 ± 0.91 ^{ab}	33.62 ± 7.87 ^{ab}	11.02 ± 3.79 ^a	57.03 ± 5.82 ^b
750	54.92 ± 3.34 ^{ab}	29.83 ± 7.78 ^{ab}	62.46 ± 8.11 ^b	51.38 ± 9.09 ^{ab}	24.90 ± 2.94 ^{ab}	68.56 ± 8.25 ^b
1000	66.91 ± 1.87 ^{ab}	41.68 ± 6.84 ^{ab}	74.57 ± 7.83 ^b	67.35 ± 11.46 ^{ab}	35.71 ± 5.33 ^{ab}	74.73 ± 7.39 ^b
1500	79.29 ± 2.64 ^{ab}	56.43 ± 4.92 ^{ab}	81.63 ± 9.11 ^b	82.95 ± 10.03 ^b	42.93 ± 4.74 ^a	87.37 ± 9.22 ^b

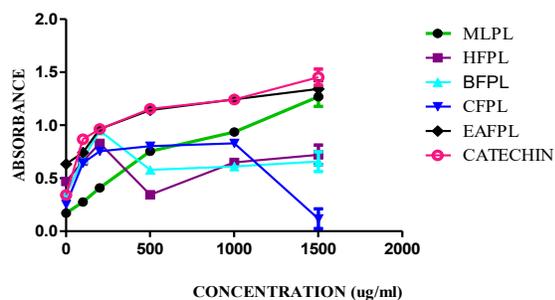
IC ₅₀ ⁺⁺	649.20	467.20	509.62	774.48	1435.05	369.04
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MLPL- Methanol leaf extract of *Periscopsis laxiflora*, HFPL- Hexane fraction of Methanol leaf extract of *Periscopsis laxiflora*, BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*, CFPL- Chloroform fraction of Methanol leaf extract of *Periscopsis laxiflora*, EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*, ^a -Significantly different from the catechin at P<0.05, ^b - Significantly increment at P<0.05 in a dose dependent manner

Table 3: Inhibitory activity of the Crude and Fractions of Methanol extract of *Periscopsis laxiflora* leaf against Malonaldehyde (Product of Lipid peroxidation) radicals relative to Catechin

CONC. (mg/mL)	% INHIBITION OF MDA					
	MLPL	HFPL	BFPL	EAFPL	CFPL	CATECHIN
Control	0.000	0.000	0.0000	0.000	0.000	0.000
100	-13.54 ± 0.67 ^{ab}	5.23 ± 1.32 ^{ab}	22.36 ± 4.37 ^{ab}	22.76 ± 4.45 ^{ab}	-9.87 ± 3.45 ^{ab}	34.83 ± 4.56 ^b
200	-05.23 ± 1.23 ^a	12.43 ± 3.54 ^{ab}	18.56 ± 3.81 ^a	42.87 ± 6.95 ^{ab}	-24.11 ± 4.81 ^{ab}	38.23 ± 2.48 ^b
500	03.65 ± 0.91 ^{ab}	19.23 ± 2.67 ^{ab}	23.11 ± 2.85 ^a	54.58 ± 5.97 ^{ab}	6.40 ± 6.79 ^{ab}	41.99 ± 5.12 ^b
750	18.01 ± 2.26 ^{ab}	43.23 ± 4.76 ^b	37.71 ± 6.21 ^{ab}	65.98 ± 5.75 ^{ab}	21.87 ± 5.85 ^{ab}	49.23 ± 5.54 ^b
1000	34.48 ± 1.97 ^{ab}	58.32 ± 8.21 ^{ab}	51.87 ± 10.12 ^{ab}	74.98 ± 4.83 ^{ab}	43.91 ± 7.12 ^{ab}	66.21 ± 4.65 ^b
1500	51.65 ± 3.72 ^{ab}	74.27 ± 7.38 ^{ab}	55.37 ± 8.05 ^a	81.45 ± 5.83 ^{ab}	64.27 ± 8.38 ^{ab}	83.39 ± 6.17 ^b
IC ₅₀ ⁺⁺	706.21	961.37	1186.75	491.52	423.85	612.32

MLPL- Methanol leaf extract of *Periscopsis laxiflora*, HFPL- Hexane fraction of Methanol leaf extract of *Periscopsis laxiflora*, BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*, CFPL- Chloroform fraction of Methanol leaf extract of *Periscopsis laxiflora*, EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*, ^a -Significantly different from the catechin at P<0.05, ^b - Significantly increment at P<0.05 in a dose dependent manner



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Figure 1: Iron II-Reducing capabilities of Crude and Fractions of *Periscopsis laxiflora* Leaf extracts.

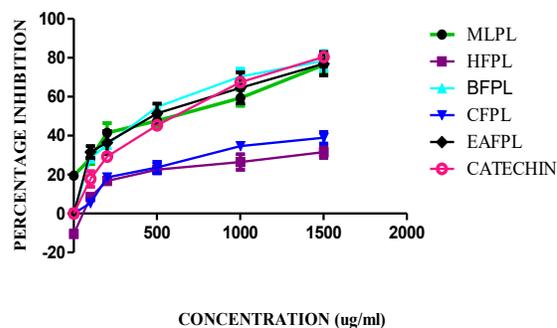
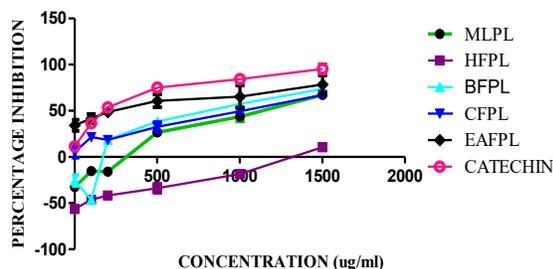


Figure 2: Inhibitory activity of the Crude and Fractions of Methanol extract of *Pericopsis laxiflora* leaf against Nitric Oxide radicals relative to Catechin
 MLPL- Methanol leaf extract of *Periscopsis laxiflora*,
 HFPL- Hexane fraction of Methanol leaf extract of *Periscopsis laxiflora*,
 BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*,
 CFPL- Chloroform fractoin of Methanol leaf extract of *Periscopsis laxiflora*,
 EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*.

Figure 3: Inhibitory activity of the Crude and Fractions of Methanol extract of *Pericopsis laxiflora* leaf against Superoxide radicals relative to Catechin
 MLPL- Methanol leaf extract of *Periscopsis laxiflora*,
 HFPL- Hexane fraction of Methanol leaf extract of *Periscopsis laxiflora*,
 BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*,
 CFPL- Chloroform fractoin of Methanol leaf extract of *Periscopsis laxiflora*,
 EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*.

Table 4: Antimicrobial activities (through inhibitory activity against some bacteria) of the Crude and fractions of Methanol extract of *Pericopsis laxiflora* (MLPL) Leaf

Concentration (mg/dL)	Micro-organisms	ZONE OF INHIBITION			
		Control (Augmentin)	MLPL	BFPL	EAFPL
100	<i>Staphylococcus aureus</i>	28.50 ± 1.33	24.50 ± 1.31 ^a	20.20 ± 1.03 ^a	26.50 ± 1.56
	<i>Streptococcus pneumoniae</i>	23.50 ± 0.76	28.50 ± 1.60 ^a	22.40 ± 0.90	22.60 ± 1.05
	<i>Escherichia coli</i>	25.50 ± 1.68	22.50 ± 1.10 ^a	24.50 ± 0.84	19.40 ± 0.54 ^a
	<i>Pseudomonas aeruginosa</i>	21.20 ± 2.33	20.50 ± 0.84	15.80 ± 0.54 ^a	15.80 ± 0.54 ^a
50	<i>Staphylococcus aureus</i>	16.00 ± 2.20 ^b	16.50 ± 0.50 ^b	19.60 ± 0.92 ^a	17.50 ± 0.75 ^b
	<i>Streptococcus pneumoniae</i>	14.00 ± 0.93 ^b	18.50 ± 1.00 ^{ab}	17.20 ± 1.25 ^{ab}	19.50 ± 1.05 ^{ab}
	<i>Escherichia coli</i>	15.00 ± 1.21 ^b	14.80 ± 0.90 ^b	13.60 ± 1.10 ^{ab}	14.00 ± 0.65 ^b
	<i>Pseudomonas aeruginosa</i>	18.00 ± 0.74 ^b	15.70 ± 0.50 ^b	13.50 ± 0.80 ^{ab}	14.50 ± 1.00 ^a
25	<i>Staphylococcus aureus</i>	10.00 ± 0.47 ^b	11.40 ± 0.54 ^b	16.50 ± 1.25 ^{ab}	11.50 ± 1.35 ^b
	<i>Streptococcus pneumoniae</i>	10.00 ± 0.72 ^b	08.30 ± 0.70 ^{ab}	14.50 ± 1.10 ^{ab}	12.00 ± 0.80 ^{ab}
	<i>Escherichia coli</i>	08.00 ± 0.91 ^b	08.80 ± 0.80	10.00 ± 0.80	07.50 ± 0.80

12.5	<i>Pseudomonas aeruginosa</i>	09.00 ± 0.74 ^b	0.22 ^b 07.00 ± 0.35 ^{ab}	0.67 ^{ab} 08.20 ± 0.65 ^b	0.50 ^b 07.00 ± 0.20 ^{ab}
	<i>Staphylococcus aureus</i>	07.00 ± 0.65 ^b	05.40 ± 0.70 ^{ab}	12.22 ± 1.00 ^{ab}	10.25 ± 0.60 ^a
	<i>Streptococcus pneumoniae</i>	08.00 ± 0.42 ^b	04.80 ± 0.44 ^{ab}	08.00 ± 0.84 ^b	05.50 ± 0.65 ^{ab}
	<i>Escherichia coli</i>	07.00 ± 0.81 ^b	06.40 ± 0.30 ^b	07.50 ± 0.60 ^b	06.00 ± 0.20
	<i>Pseudomonas aeruginosa</i>	08.00 ± 0.79 ^b	08.00 ± 0.79	05.40 ± 0.23 ^{ab}	06.00 ± 0.45 ^a

MLPL- Methanol leaf extract of *Periscopsis laxiflora*, BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*, EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*. ^a -Significantly different from the control at P<0.05, ^b - Significantly reduction at P<0.05 in a dose dependent manner.

Table 5: Antimicrobial activities (through inhibitory activity against some fungi) of the Crude and fractions of Methanol extract of *Periscopsis laxiflora* (MLPL) Leaf

Concentration (mg/dL)	Micro-organisms	ZONE OF INHIBITION			
		Control (Griseofulvin)	MLPL	BFPL	EAFPL
100	<i>Candida albicans</i>	30.20 ± 2.41	29.40 ± 3.00	22.50 ± 1.82 ^a	24.00 ± 1.00 ^a
	<i>Aspergillus spp</i>	21.60 ± 1.22	23.00 ± 2.20	15.26 ± 1.40 ^a	10.30 ± 1.20 ^a
50	<i>Candida albicans</i>	24.10 ± 2.61 ^b	25.40 ± 1.26 ^b	19.40 ± 0.84 ^{ab}	22.50 ± 2.40 ^b
	<i>Aspergillus spp</i>	15.20 ± 0.80 ^b	16.50 ± 1.47 ^b	11.50 ± 1.26 ^{ab}	08.00 ± 0.60 ^{ab}
25	<i>Candida albicans</i>	20.22 ± 1.20 ^b	19.50 ± 1.00 ^b	14.50 ± 1.70 ^{ab}	19.00 ± 1.60 ^b
	<i>Aspergillus spp</i>	13.00 ± 0.60 ^b	15.20 ± 2.00 ^b	08.70 ± 0.65 ^{ab}	05.10 ± 0.45 ^{ab}
12.5	<i>Candida albicans</i>	14.40 ± 0.42 ^b	14.00 ± 1.40 ^b	10.45 ± 1.30 ^{ab}	15.50 ± 0.66 ^b
	<i>Aspergillus spp</i>	10.00 ± 0.56 ^b	11.00 ± 0.50 ^b	06.50 ± 0.20 ^{ab}	02.00 ± 0.05 ^{ab}

MLPL- Methanol leaf extract of *Periscopsis laxiflora*, BFPL- Butanol fraction of Methanol leaf extract of *Periscopsis laxiflora*, EAFPL- Ethylacetate fraction of Methanol leaf extract of *Periscopsis laxiflora*. ^a - Significantly different from the control at P<0.05, ^b - Significantly reduction at P<0.05 in a dose dependent manner.

IV. DISCUSSION

The result of the phytochemical screening (Table 1) depicted clear variations in the distribution of secondary metabolites among the crude *Methanol* extract and the different solvent fractions of *Periscopsis laxiflora* leaf. The *Methanol* leaf extract (MLPL) exhibited the widest range of

phytochemicals, including flavonoids, alkaloids, saponins, phlobatannins, and anthraquinones. The hexane fraction (HFPL) showed a more limited phytochemical profile, with the presence of alkaloids and saponins but the absence of most other constituents. This observation may be attributed to the non-polar nature of hexane, which favors the extraction of lipophilic compounds while excluding

more polar phytochemicals such as flavonoids and tannins. The butanol fraction (BFPL) contained anthraquinones, flavonoids, and phlobatannins, but lacked alkaloids, saponins, tannins, and cardiac glycosides. Butanol, being moderately polar, appears to selectively concentrate certain phenolic and quinone-based compounds.

In contrast, the chloroform fraction (CFPL) revealed the presence of anthraquinones, flavonoids, alkaloids, and tannins, indicating a relatively diverse phytochemical composition. Chloroform is known to extract compounds of low to medium polarity, which may explain the recovery of both alkaloids and phenolic compounds in this fraction. The ethyl acetate fraction (EAFPL) showed the presence of saponins, phlobatannins, and tannins but lacked flavonoids, alkaloids, and anthraquinones. This pattern highlights the ability of ethyl acetate to concentrate certain phenolic and glycosidic compounds. Notably, cardiac glycosides were absent across all extracts and fractions, indicating that *P. laxiflora* leaves may not be a significant source of this class of phytochemicals.

The phytochemical profile of *Periscopsis laxiflora* leaf extracts therefore suggests considerable medicinal potential, as several bioactive compounds of known therapeutic value were detected across the crude extract and solvent fractions. The presence of flavonoids in the *Methanol*, butanol, ethyl acetate, and hydro-ethanol fractions indicate possible antioxidant, anti-inflammatory, and antimicrobial activities, which support the traditional use of the plant in managing infections and inflammatory conditions. Alkaloids, observed in the *Methanol*, hexane, ethyl acetate, and hydro-ethanol fractions, are widely associated with analgesic, antimalarial, and antimicrobial properties, further highlighting the pharmacological relevance of the plant. Saponins and tannins, detected in selected fractions, are known for their antimicrobial, wound-healing, and anti-diarrheal effects, while the presence of anthraquinones suggests potential laxative and antimicrobial actions. Although cardiac glycosides were absent, the overall diversity of secondary metabolites indicates that *P. laxiflora* leaves are a rich source of medicinally important compounds and justify further

pharmacological and toxicological investigations to validate their therapeutic applications.

Table 2 and 3 depicted the result of DPPH radical scavenging and lipid peroxidation (MDA) inhibition assays, respectively, of crude methanol extract and its fractions of *Pericopsis laxiflora* leaf. In the DPPH assay, all samples except the hexane and chloroform fractions at lower concentrations demonstrated progressive increases in radical scavenging activity with increasing concentration. The hexane fraction relatively has poor performance suggests a low abundance of effective radical-scavenging compounds in it. A similar pattern was observed in the lipid peroxidation inhibition assay. The ethyl acetate fraction consistently showed a better inhibition of malonaldehyde formation across all tested concentrations, with a maximum inhibition of 81.45% at 1500 mg/mL and a relatively low IC₅₀ value (491.52 mg/mL), indicating strong antioxidant efficiency. The hexane fraction exhibited improved activity only at higher concentrations, while the crude *Methanol* extract and butanol fraction showed moderate but consistent inhibition. The chloroform fraction, although less effective at lower concentrations, displayed improved activity at higher doses, suggesting the presence of compounds with weaker but cumulative antioxidant effects.

Based on both assays and IC₅₀ values, the ethyl acetate fraction (EAFPL) can be identified as the extract with the best overall antioxidant activity. Although the butanol fraction showed slightly higher DPPH scavenging at the highest concentration, EAFPL demonstrated more consistent performance across both DPPH and lipid peroxidation assays and possessed lower IC₅₀ values in the MDA model, indicating greater potency at lower concentrations relative to the standard, Catechin. This present findings on the *Pericopsis laxiflora* extracts align well with recent research trends showing that plant extracts rich in phenolic compounds and flavonoids tend to exhibit strong antioxidant effects. For example, a hydroethanolic extract of *P. laxiflora* roots was previously shown to contain high levels of phenols, flavonoids, tannins, and other bioactive compounds, and this phytochemical richness contributed to measurable biological activity in vivo, which indirectly supports the idea that phenolic-rich

extracts have significant functional properties (Ouattara *et al.*, 2025). Similarly, a recent study on other medicinal herbs found that higher phenolic and flavonoid contents were closely correlated with stronger free-radical scavenging activity in DPPH and other antioxidant assays (Oyedemi *et al.*, 2025). These collective observations corroborate the present results in which the ethyl acetate and butanol fractions, both shown earlier to be rich in flavonoids, tannins, and anthraquinones, demonstrated the best antioxidant performance across both DPPH and lipid peroxidation assays.

The reducing power assay presented in Figure 1 demonstrates that the crude *Methanol* extract and its solvent fractions of *Pericopsis laxiflora* leaf possess varying capacities to donate electrons, which is a key mechanism underlying antioxidant activity. A clear concentration-dependent increase in reducing capability was observed across all extracts except Hexane and chloroform fraction, indicating their potential to neutralize oxidized intermediates. Among the samples, the ethyl acetate fraction (EAFPL) and butanol fraction (BFPL) consistently showed stronger reducing power relative to the standard, catechin.

Figures 2 and 3 further illustrate the antioxidant potential of the extracts through nitric oxide and superoxide radical scavenging assays, respectively. In both models, the extracts demonstrated appreciable radical inhibition in a dose-dependent manner, with marked differences among fractions. The ethyl acetate and butanol fractions again showed superior scavenging of nitric oxide and superoxide radicals, closely approaching the activity of the reference antioxidant, catechin. In contrast, the hexane fraction displayed relatively low scavenging activity, particularly at lower concentrations, indicating limited effectiveness against reactive nitrogen and oxygen species. Overall, the ethyl acetate fraction (EAFPL) and Butanol fraction (BFPL) emerged as the extract with the best antioxidant activity across the reducing power, nitric oxide, and superoxide radical assays. Their consistent performance in different antioxidant models suggests the presence of a broad range of bioactive compounds capable of acting through multiple antioxidant mechanisms. The activities are well comparable with the Standard and

the crude methanol extract and may be due to their mixed phytochemical composition.

Thereafter, the two most active fractions of the methanol extract of *Pericopsis laxiflora* leaf (MLPL), ethyl acetate fraction (EAFPL) and butanol fraction (BFPL) were further subject to antimicrobial activities and the findings were depicted in Table 4 and 5. In table 4, the MLPL and its fractions possess appreciable inhibitory effects against both bacterial and fungal pathogens accommodated in this study, although the degree of activity varies with extract type, microorganism, and concentration. At the highest concentration (100 mg/dL), MLPL showed strong activity against *Staphylococcus aureus* and *Streptococcus pneumoniae*, with zones of inhibition close to the standard antibiotic (Augmentin) for some pathogens. The ethyl acetate fraction (EAFPL) similarly demonstrated notable inhibitory effects, particularly against *Staphylococcus aureus* at higher concentrations, while the butanol fraction (BFPL) showed relatively moderate antibacterial activity. Among the tested bacterial strains, *S. pneumoniae* was particularly sensitive to MLPL, suggesting that the crude extract contains bioactive phytochemicals capable of targeting respiratory pathogens.

Against fungal organisms (Table 5), the crude extract again showed meaningful activity. MLPL and EAFPL produced inhibition zones against *Candida albicans* comparable to the control antifungal (griseofulvin) at the highest dose, underscoring the broad spectrum of antimicrobial properties present in the leaf extract. The butanol fraction, while active, generally exhibited smaller zones of inhibition against both *Candida* and *Aspergillus* species compared to MLPL and EAFPL. At lower concentrations, the decline in zone size across all extracts and microorganisms indicates a dose-dependent response, which is characteristic of antimicrobial agents and supports the reliability of these results.

Overall, the ethyl acetate fraction (EAFPL) displayed the best antimicrobial activity when considering both antibacterial and antifungal performance across multiple organisms, closely followed by the crude *Methanol* extract. This aligns with the phytochemical profile discussed earlier, where EAFPL was rich in

flavonoids, anthraquinones, and tannins, compounds well-documented for their antimicrobial properties. Flavonoids and tannins are known to disrupt microbial cell walls and interfere with enzyme systems, which likely accounts for the observed inhibition of both bacterial and fungal pathogens. The stronger activity in the ethyl acetate fraction underscores the importance of moderately polar phytochemicals in combating microbial infections.

With rising resistance to conventional antibiotics and antifungals, plant-derived extracts with broad-spectrum antimicrobial activity offers promising alternatives or adjuncts to existing therapies. The ability of *P. laxiflora* extracts, especially EAFPL, to inhibit common pathogens such as *S. aureus*, *S. pneumoniae*, and *C. albicans* suggests potential utility in the development of natural antimicrobial agents for treating respiratory, skin, and opportunistic infections. These findings correlate with recent research on *Pericopsis laxiflora* and related medicinal plants, which supports the presence of antimicrobial effects associated with phenolic compounds and flavonoids. For example, ethnobotanical reviews have highlighted the use of *P. laxiflora* in traditional medicine for treating infections and gastrointestinal ailments (Sarfo-Antwi *et al.*, 2021). Experimental investigations have also demonstrated that extracts of *P. laxiflora* exhibit antibacterial and antifungal effects *in vitro*, consistent with the current results (Doukourou *et al.*, 2025; Oyedeji *et al.*, 2025). Moreover, studies have found that plant fractions rich in polyphenols and flavonoids tend to exhibit higher antimicrobial activity compared to non-polar fractions, mirroring the superior performance of the ethyl acetate fraction in this study (Rizliya Visvanathan *et al.*, 2025). Together, these references reinforce the view that the antimicrobial efficacy observed here is attributable to the phytochemical composition of the extracts and is in line with contemporary research findings.

V. CONCLUSION

The present study demonstrates that the methanol extract and fractions of *Pericopsis laxiflora* leaf displayed significant *in vitro* antioxidant and antimicrobial activities, with the ethyl acetate fraction (EAFPL) consistently exhibiting the highest potency

across multiple assays. Phytochemical analysis revealed the presence of flavonoids, anthraquinones, tannins, saponins, and alkaloids, which likely contribute to the observed bioactivities. EAFPL's strong radical scavenging, reducing power, and inhibition of lipid peroxidation suggest its potential in mitigating oxidative stress, while its broad-spectrum antibacterial and antifungal effects indicate promising applications against pathogenic microorganisms. The study highlights the importance of bioassay-guided fractionation in identifying the most bioactive plant components, providing a scientific basis for the traditional use of *P. laxiflora* in managing infections and oxidative stress-related conditions. These findings warrant further *in vivo* and clinical investigations to evaluate safety, efficacy, and therapeutic potential.

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