

Phytochemical Composition, Antioxidant Activity and Antimicrobial Properties of the Methanolic Extract and Solvent Fractions of *Ritchiea Longipedicellata*

¹Sunday Felix Abimbade, ¹Olalekan Cosmas Ogundola, ¹Kanayo Samuel Okonji, ^{*1}Oluwabamise Lekan Faboya and ²Ayodeji Paul Awonegan

¹Department of Chemistry, Faculty of Physical Sciences, Federal University Oye-Ekiti, Ekiti State, Nigeria.

²Department of Science Technology, School of Science and Computer Studies, The Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria.

*Corresponding authors' email: lekan.faboya@fuoye.edu.ng

ABSTRACT

Antimicrobial resistance continues to pose a serious threat to global health, driving the search for new bioactive compounds from natural plant sources. *Ritchiea longipedicellata* (Capparaceae), traditionally used in West Africa to treat infectious diseases, remains poorly characterized in medicinal chemistry. This study investigated the phytochemical content, antioxidant activity, and antimicrobial properties of hexane and chloroform fractions from a methanolic extract of *R. longipedicellata*'s aerial parts, collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan. Aerial parts were air-dried, pulverized, macerated in methanol, and sequentially partitioned with hexane and chloroform. Qualitative screening confirmed alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, diterpenoids, and cardiac glycosides in both fractions. Quantitative analysis showed the hexane fraction had higher phenolic (189.98 ± 0.28 mg GAE/g) and flavonoid (32.41 ± 0.42 mg QE/g) content than the chloroform fraction. DPPH assays showed good antioxidant activity for the crude extract, hexane, and chloroform fractions (81.06%, 84.20%, and 77.98% inhibition at 500 μ g/mL) versus 96.40% for ascorbic acid, with dose-dependent effects. Antibacterial and antifungal MIC values across the tested extracts and organisms ranged from 1.5625 mg/mL to >50 mg/mL, with the chloroform fraction consistently the most active, achieving MICs of 1.5625 mg/mL against both *Staphylococcus aureus* and *Escherichia coli*. MBC/MFC values (3.125–50 mg/mL) confirmed bactericidal or fungicidal activity at higher concentrations for several extract–organism combinations, though extract MICs remained substantially higher than that of ciprofloxacin. *Candida albicans* and *Trichophyton rubrum* were also susceptible to the chloroform fraction, with MICs of 12.5 mg/mL and 3.125 mg/mL, respectively. These findings indicate that *R. longipedicellata* contains bioactive constituents of interest for further phytochemical and antimicrobial investigation, warranting compound isolation and additional biological evaluation before any therapeutic application can be inferred.

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INTRODUCTION

Antimicrobial resistance is now one of the defining public-health threats of this century. Bacterial antimicrobial resistance (AMR) was directly responsible for an estimated 1.27 million deaths worldwide in 2019 and contributed to a further 3.68 million, a burden that in that year exceeded deaths from either HIV/AIDS or malaria, with the highest mortality rates recorded in sub-Saharan Africa (Antimicrobial Resistance Collaborators, 2022). This burden continues to grow: resistant infections now threaten the effective treatment of an ever-increasing range of common bacterial, parasitic, and fungal diseases worldwide (WHO, 2023). Alongside this infectious-disease burden, oxidative stress, a normal metabolic process that becomes pathological once it outpaces antioxidant defences, is implicated in the pathogenesis of cardiovascular disease, diabetes and several cancers, non-communicable diseases whose prevalence is also rising across West Africa. A plant fraction combining antimicrobial and antioxidant activity is therefore of interest as a candidate for further pharmacological investigation relevant to both disease categories.

Medicinal plants have served as a rich source of bioactive molecules that may lead to the development of new or adjunct therapies to overcome the problem of drug-resistant microbes (Cowan, 1999), and natural products and their derivatives still occupy a significant percentage of the market share of new drugs across practically all therapeutic areas (Newman & Cragg, 2020). Among West African flora, members of the genus *Ritchiea*, family Capparaceae (formerly Capparidaceae), have been extensively used in the treatment of infectious and parasitic diseases by traditional medicine practitioners in Nigeria (Taiwo et al., 2013). This is in agreement with general ethnobotanical reports detailing the medicinal properties and ethnobotany of West African vegetation (Burkill, 1985; Sofowora, 1993). A quaternary ammonium compound, a levo-isomer of stachydrine, has been shown to exhibit antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* in *Ritchiea capparoides* var. *longipedicellata* (Taiwo et al., 2013). Separately, a phytochemical analysis of the root bark of the same species, a different plant part from the aerial parts examined in the present study, showed the presence of

alkaloids, flavonoids, triterpenes and glycosides (Anowi et al., 2013). Quantitative data on the constituents of the aerial parts, and scientific validation of the plant's ethnomedicinal uses, remain scarce. *Ritchiea longipedicellata* is among the least studied species in the genus, and although its medicinal value is recognised by indigenous populations, an adequate quantitative account of its phytochemical profile and pharmacological activity is still lacking in the scientific literature.

This study therefore aimed at elucidating the quantitative phytochemical profile, including total phenolic, flavonoid, alkaloid, saponin, and tannin content, of the hexane and chloroform fractions derived from the methanolic extract of the aerial parts of *R. longipedicellata*, and at evaluating the antioxidant and antimicrobial activity of these fractions against a panel of clinically relevant Gram-positive and Gram-negative bacteria and fungi. By quantifying, rather than merely detecting, the major classes of secondary metabolites present, and by benchmarking antioxidant and antimicrobial performance directly against ascorbic acid and standard antibiotics, this work aims to move the evidence base for *R. longipedicellata* beyond qualitative ethnobotanical claims and toward quantitative, comparable data that can justify further isolation and pharmacological development work.

MATERIALS AND METHODS

Plant Material Collection and Identification

Aerial parts of *Ritchiea longipedicellata* were collected during the flowering season from Ibadan in the south-western part of Nigeria in June 2025. The plant material was identified and authenticated by Mr. Adedeji, a qualified botanist at the Herbarium Section, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, where a voucher specimen was deposited (voucher number: 117251).

Preparation of Methanolic Extract and Fractionation

The collected aerial parts were thoroughly washed and air-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10 days. The dried sample was ground into a coarse powder using a mechanical grinder. Approximately 500 g of the powdered material was macerated with 2 L of methanol (analytical grade) at room temperature for 72 hours with occasional stirring. The extract was filtered through Whatman No. 1 filter paper. The solvent was thereafter removed under reduced pressure using a rotary evaporator at 40°C to yield 300 g of crude methanolic extract (60% w/w yield relative to the starting dry plant mass).

The crude extract was suspended in methanol and subjected to successive liquid-liquid partitioning, first with 500 mL of hexane (three extraction cycles) and then with 500 mL of chloroform (three extraction cycles), to obtain the respective fractions. Each fraction was concentrated under reduced pressure and stored at 4°C for further analysis, giving a hexane fraction yield of 35.2% w/w (105.6 g) and a chloroform fraction yield of 26.5% w/w (79.5 g), each relative to the 300 g crude extract. The polar residue remaining in the mother liquor after sequential removal of the hexane- and chloroform-soluble material is hereafter referred to as the "methanol" fraction in Table 1 and is distinct from the original crude methanolic extract; it accounted for the remaining 38.3% w/w (114.9 g) of the crude extract mass.

Phytochemical Screening

Preliminary phytochemical screening of hexane and chloroform fractions was performed using standard qualitative methods to detect classes of bioactive compounds such as alkaloids, flavonoids, phenolics, terpenoids, saponins, and glycosides (Harborne, 1973). These procedures follow

established protocols widely used for phytochemical screening and extraction of plant secondary metabolites (Trease & Evans, 2002; Tiwari et al., 2011). All quantitative phytochemical determinations described below were performed independently on the crude, hexane, chloroform and methanol preparations. All quantitative determinations were performed in triplicate ($n = 3$), and results are presented as mean $\pm$ standard deviation.

Alkaloid Determination (Harborne, 1973)

5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Saponin Determination

The method used was that of Obadoni and Ochuko (2002). The samples were ground and 20 g of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hrs with continuous stirring at about 55°C . The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C . The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the Saponin content was calculated as percentage.

Determination of Total Phenolic Content

The concentration of phenolic in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 mL of extract and 9 mL of distilled water was taken in a volumetric flask (25 mL). One milliliter of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 mL of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 mL. A set of standard solutions of Gallic acid (20, 40, 40, 60, 80 and 100 $\mu\text{g/mL}$) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV /Visible spectrophotometer). Total phenol content was expressed as mg of GAE/gm of extract.

Determination of Tannin Content

The tannins were determined by Folin-Ciocalteu method. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu Phenol reagent, 1 mL of 35 % Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 $\mu\text{g/mL}$) were prepared in the same manner as

described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

Determination of Total Flavonoid Content

Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1 mL of extract and 4 mL of distilled water was taken in a 10 mL volumetric flask. To the flask, 0.30 mL of 5 % sodium nitrite was treated and after 5 minutes, 0.3 mL of 10 % aluminum chloride was mixed. After 5 minutes, 2 mL of 1M Sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of Quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions was determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Antimicrobial Activity

Microbial Strains

The antimicrobial activity of the fractions was tested against a panel of microorganisms comprising Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (*Candida albicans*, *Trichophyton rubrum*).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the fractions was determined using the broth microdilution technique according to the Clinical and Laboratory Standards Institute (CLSI) M07 guideline, generally in keeping with standard methods used for determining the antibacterial activity of phytochemicals derived from plants (Ncube et al., 2008). Stocks of the fractions were first constituted with dimethyl sulfoxide (DMSO) and serially diluted in Mueller–Hinton broth or Sabouraud dextrose broth for the bacteria or fungi, respectively, to a final concentration range of 0.0305–50 mg/mL.

Each microbial strain or isolate tested was identified. The isolates were not subjected to formal antimicrobial-resistance phenotyping as part of the present study; therefore, their specific resistance profiles were not assigned.

Microbial suspensions adjusted to a 0.5 McFarland standard (approximately 1×10^8 CFU/mL) were inoculated into each well to a final inoculum density of approximately 5×10^5 CFU/mL. Plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi. The MIC was recorded as the lowest concentration of the fraction that visibly inhibited microbial growth. DMSO ($\leq 1\%$) and standard antimicrobial agents (ciprofloxacin for bacteria and ketoconazole for fungi) were included as negative (vehicle) and positive controls, respectively, alongside broth-only sterility and organism-only growth controls (Table 2).

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Following MIC determination, an aliquot (10 µL) was withdrawn from each well showing no visible growth and

subcultured onto antibiotic-free Mueller-Hinton (for bacteria) or Sabouraud dextrose (for fungi) agar, then incubated under the same temperature and time conditions as above. The MBC (for bacteria) or MFC (for fungi) was recorded as the lowest concentration yielding no visible colony growth on subculture ($\geq 99.9\%$ kill relative to the inoculum), consistent with the endpoint convention used in plant-extract susceptibility testing (Eloff, 2019)

DPPH Antioxidant Analysis

Procedure Adapted from Brand-Williams et al. (1995), with Modifications by Manzocco et al. (1998)

The molecule 1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of the electron also gives rise to a deep violet colour, characterized by an absorption band in methanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, this gives rise to the reduced form with loss of the violet colour. To evaluate antioxidant potential through free-radical scavenging, the change in optical density of DPPH radicals was monitored at sample concentrations of 31.25–500 µg/mL for the crude extract, hexane fraction, chloroform fraction and ascorbic acid standard. Each concentration was analysed in triplicate ($n = 3$), and the results were expressed as mean $\pm$ standard deviation. Following Manzocco et al. (1998), with slight modification of Brand-Williams et al. (1995), 1 mL of sample extract was mixed with 1 mL of DPPH solution (0.3 mM). After 30 min in the dark, the absorbance was measured at 517 nm. The percentage DPPH radical-scavenging activity was calculated as: % inhibition = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} is the absorbance of the DPPH solution without sample and A_{sample} is the absorbance after reaction with the sample. An IC₅₀ estimate derived from the concentration–response curves would provide an additional, more directly comparable measure of antioxidant potency.

Statistical Analysis

All quantitative determinations were performed in triplicate ($n = 3$) and are expressed as mean $\pm$ standard deviation. Group differences were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple-comparison test, with statistical significance set at $p < 0.05$. Data were analysed using IBM SPSS Statistics version 29.0.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Qualitative phytochemical evaluation for the crude, hexane, chloroform and methanol fractions of *Ritchiea longipedicellata* found that all fractions contained saponins, alkaloids, flavonoids, tannins, phenolic compounds, coumarins, terpenoids, steroids, diterpenes and cardiac glycosides (Table 1). The presence of such an array of secondary metabolites in different solvent fractions indicates that the phytochemical profile of the plant is diverse, and each type may possess different biological activity (Harborne, 1973).

Table 1: Qualitative Phytochemical Screening of Crude, Hexane, Chloroform, and Methanol Fractions of the Aerial Parts of *Ritchiea Longipedicellata*

Parameter	R. Longipedicellata Crude	R. Longipedicellata Hexane	R. Longipedicellata Chloroform	R. Longipedicellata Methanol
Saponin (Froth's Test)	+	+	+	+
Alkaloid (Hager's Test)	+	+	+	+
Flavonoid (Lead acetate Test)	+	+	+	+
Tannin (Braymer's Test)	+	+	+	+
Phenol (Ferric Chloride Test)	+	+	+	+
Coumarin (Reaction with 10 % NaOH)	+	+	+	+
Terpenoid (Salkowski's test)	+	+	+	+
Steroid (Salkowski's Test)	+	+	+	+
Diterpenes (Copper acetate test)	+	+	+	+
Cardiac Glycosides (Keller kiliani Test)	+	+	+	+

“+” indicates the phytochemical class was detected. Crude = crude methanolic extract (300 g, 60% w/w yield from 500 g dry plant material); Hexane and Chloroform = solvent-partitioned fractions of the crude extract (35.2% and 26.5% w/w yield, respectively); Methanol = the polar residue remaining after sequential removal of the hexane- and chloroform-soluble material from the crude extract (38.3% w/w yield)

Quantitative Phytochemical Screening

Quantitative phytochemical analysis showed clear differences among the crude, hexane and chloroform preparations (Figures 1 and 2). The crude extract contained alkaloid (mean $14.75 \pm 0.40\%$), flavonoid (28.89 ± 0.28 mg QE/g), phenolic (111.20 ± 0.77 mg GAE/g), saponin ($5.60 \pm 0.22\%$) and tannin (8.44 ± 0.09 mg GAE/g) content. The hexane fraction had the highest phenolic (189.98 ± 0.28 mg

GAE/g) and flavonoid (32.41 ± 0.42 mg QE/g) content among the three preparations, but lower alkaloid ($5.55 \pm 0.49\%$) and saponin ($3.58 \pm 0.28\%$) content than the crude extract. The chloroform fraction had the lowest flavonoid (6.74 ± 0.18 mg QE/g), phenolic (25.83 ± 0.45 mg GAE/g), tannin (3.99 ± 0.12 mg GAE/g) and alkaloid ($8.53 \pm 0.03\%$) content of the three preparations.

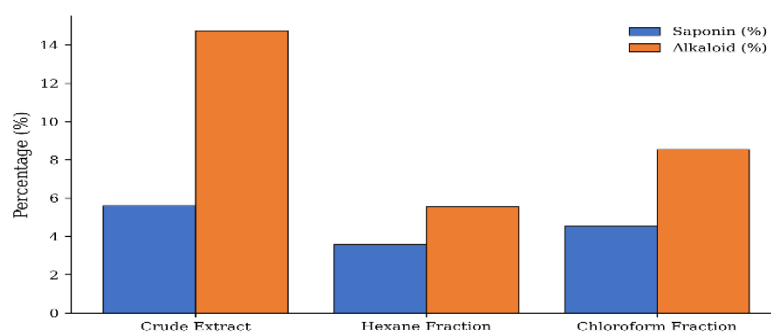


Figure 1: Quantitative alkaloid and saponin content (% w/w) of crude, hexane, and chloroform fractions of *Ritchiea longipedicellata*. Error bars represent standard deviation (SD; n = 3)

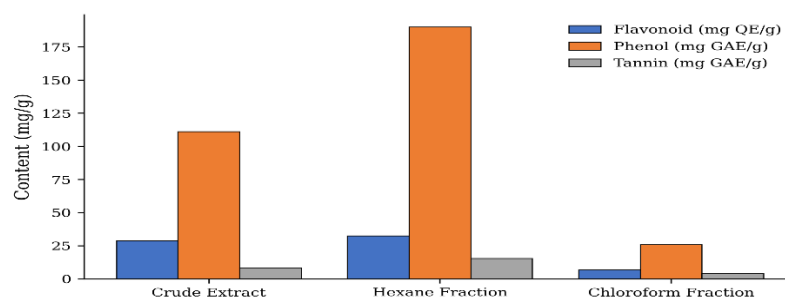


Figure 2: Total phenolic (mg GAE/g), flavonoid (mg QE/g) and tannin (mg GAE/g) content of crude, hexane, and chloroform fractions of *Ritchiea longipedicellata*. Error bars represent standard deviation (SD; n = 3)

DPPH Antioxidant Assay

The DPPH radical-scavenging activity of *R. longipedicellata* extracts is shown in Figure 3. At 500 µg/mL, ascorbic acid neutralised 96.40% of DPPH radicals. Among the extracts, the hexane fraction recorded the highest scavenging activity

(84.20%), followed by the crude extract (81.06%) and the chloroform fraction (77.98%); this ranking (hexane > crude > chloroform) was consistent across the concentration range tested (31.25–500 µg/mL), with scavenging activity increasing with concentration for all samples.

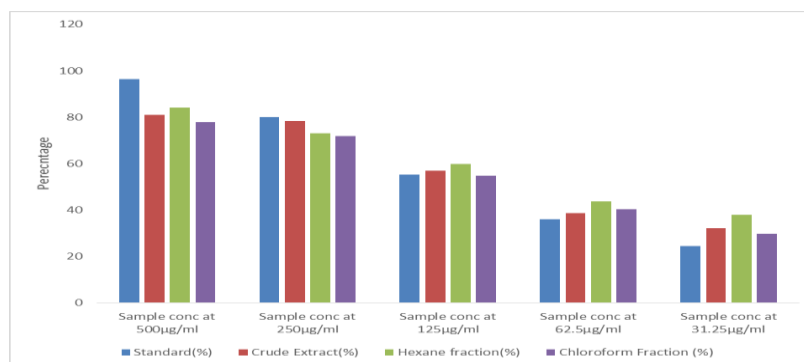


Figure 3: DPPH radical-scavenging activity (%) of crude, hexane, and chloroform fractions of *Ritchiea longipedicellata* compared with the ascorbic acid standard, at sample concentrations of 31.25–500 µg/mL. Ascorbic acid served as the positive (reference antioxidant) control. Error bars represent standard deviation (SD; n = 3)

Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC, mg/mL)

Table 2 reports the MIC and MBC/MFC values of the crude, hexane and chloroform preparations against the bacterial and fungal panel. Among the three preparations, the chloroform fraction gave the lowest (most active) MIC values against most organisms tested: 1.5625 mg/mL against both *Staphylococcus aureus* and *Escherichia coli*, 3.125 mg/mL against *Bacillus subtilis*, and 25 mg/mL against *Pseudomonas aeruginosa*. The hexane fraction was notably more active than the chloroform fraction specifically against *P. aeruginosa* (MIC 3.125 mg/mL). The crude extract had the

highest (least active) MIC values overall, generally ≥ 50 mg/mL. MBC/MFC values ranged from 3.125 to 50 mg/mL and, where determined, were within one to two two-fold dilution steps of the corresponding MIC, consistent with bactericidal or fungicidal rather than purely bacteriostatic/fungistatic action at those concentrations. By comparison, the ciprofloxacin control achieved MICs of 5–10 µg/mL and the ketoconazole control achieved MICs of 1% against the fungal panel, both several orders of magnitude more potent (i.e., effective at much lower concentrations) than any plant preparation tested.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Mycidal Concentration (MBC/MFC), in mg/mL, of *Ritchiea Longipedicellata* Crude Extract and Hexane and Chloroform Fractions against Selected Bacterial and Fungal Strains, with Ciprofloxacin (µg/mL) and Ketoconazole (% w/v) as Standard Controls. “+” = Growth Observed; “–” = No Growth; NA = Not Applicable (Control not Tested against that Organism Class). Sterility Control = Uninoculated Broth; Solvent Control = Broth + DMSO Vehicle only, No Organism; Growth Control = Broth + Test Organism only, No Extract

Organisms	Ritchiea Longipedicellata Crude		Ritchiea Longipedicellata Hexane		Ritchiea Longipedicellata Chloroform		Sterility Control (Broth Only)	Solvent Control (Broth + DMSO)	Growth Control (Broth + Isolate)	CONTROL Ciprofloxacin (µg/mL)		CONTROL Ketoconazole (%)	
	MIC	MMC	MIC	MMC	MIC	MMC				MIC	MBC	MIC	MBC
<i>Staphylococcus aureus</i>	>50	>50	50	50	1.5625	3.125	–	–	+	NA	NA	5	5
<i>Bacillus subtilis</i>	50	50	50	50	3.125	12.5	–	–	+	NA	NA	>5	10
<i>Escherichia coli</i>	50	>50	50	50	1.5625	25	–	–	+	NA	NA	10	10
<i>Pseudomonas aeruginosa</i>	50	50	3.125	12.5	25	25	–	–	+	NA	NA	>10	>10
<i>Candida albicans</i>	50	50	6.25	25	12.5	25	–	–	+	1%	0.25	NA	NA
<i>Trichophyton rubrum</i>	>50	>50	50	50	3.125	12.5	–	–	+	1%	1%	NA	NA

Discussion

Phytochemical Content

The hexane fraction's markedly higher phenolic and flavonoid content relative to the chloroform fraction is consistent with the preferential partitioning of moderately non-polar phenolics and flavonoid aglycones into hexane, while the crude extract's higher alkaloid and saponin content reflects its broader, unfractionated composition. The phenolic content of the hexane fraction (190 mg GAE/g) and the flavonoid content of the crude and hexane preparations (>20 mg QE/g) fall within ranges reported for other phenolic- and flavonoid-rich medicinal plant extracts (Prior et al., 2005; Phuyal et al., 2020), suggesting *R. longipedicellata* is

comparatively rich in these classes of secondary metabolites. Folin–Ciocalteu and aluminium chloride assays are non-specific colorimetric methods; they quantify total reducing/phenolic and flavonoid-reactive content but do not identify individual compounds, so no claim is made here about the identity of specific phenolic or flavonoid constituents. Differences in phytochemical content across fractions are consistent with differences in solvent polarity affecting which metabolite classes are extracted, and provide a basis, but not direct evidence, for the antioxidant and antimicrobial activities discussed below.

Antioxidant Activity

All three preparations showed substantial DPPH radical-scavenging activity, though consistently lower than the ascorbic acid reference standard, in line with the broad 70–90% inhibition typically reported at 500 µg/mL for plant extracts of comparable phenolic content (Brand-Williams et al., 1995; Prior et al., 2005). The hexane fraction's higher scavenging activity is consistent with its higher phenolic and flavonoid content and several compounds reported elsewhere in similarly non-polar plant fractions, including squalene (Lou-Bonafonte et al., 2018) and thymol-type monoterpenoids (Escobar et al., 2020), have documented antioxidant activity; however, no compound-identification analysis (e.g. GC-MS) was performed in this study, so their presence in *R. longipedicellata* and any contribution to the observed activity remain unconfirmed. It should also be emphasised that DPPH radical-scavenging activity is an *in vitro* chemical assay and does not, by itself, demonstrate protection against oxidative-stress-related disease in humans; extrapolation to clinical antioxidant benefit would require further *in vivo* investigation.

Antimicrobial Activity

The chloroform fraction showed the most consistent antibacterial activity of the three preparations, with MICs in the low mg/mL range against *S. aureus*, *E. coli* and *B. subtilis*, while the hexane fraction was the more active preparation against *P. aeruginosa*. Close agreement between MIC and MBC/MFC values for most extract–organism combinations suggest bactericidal or fungicidal, rather than purely inhibitory, action at the concentrations tested. Nevertheless, all plant-preparation MICs (mg/mL range) were several orders of magnitude higher than the ciprofloxacin MIC (µg/mL range), meaning the extracts are far less potent than the reference antibiotic on a mass basis; no plant preparation tested here would be expected to substitute for ciprofloxacin or ketoconazole in treating an active infection. No isolate was confirmed to carry a documented resistance phenotype, so activity “against drug-resistant organisms” cannot be claimed from these data. No cytotoxicity or mammalian-cell toxicity testing was performed, so the therapeutic window of these preparations is unknown. The relatively weaker activity of the hexane and crude preparations, despite their higher phenolic and flavonoid content, alongside the relatively stronger activity of the less phenolic-rich chloroform fraction, suggests that antimicrobial activity in *R. longipedicellata* aerial parts is not driven solely by the phenolic/flavonoid pool, supporting bioassay-guided fractionation, rather than whole-extract screening, as the appropriate next step for isolating the responsible constituents.

CONCLUSION

To our knowledge, this is the first study to provide a quantitative phytochemical profile alongside antioxidant and antimicrobial evaluation of the hexane and chloroform fractions of *R. longipedicellata* aerial parts. The tested fractions showed distinct phytochemical profiles, DPPH radical-scavenging responses and antimicrobial inhibition patterns: the hexane fraction was richer in phenolics and flavonoids and showed the highest DPPH scavenging activity, while the chloroform fraction showed the strongest and most consistent antibacterial activity against the Gram-positive and Gram-negative panel (MIC as low as 1.5625 mg/mL against *S. aureus* and *E. coli*) despite its lower phytochemical content. These findings indicate that *R. longipedicellata* is a source of quantifiable antioxidant and antimicrobial activity worth

further investigation, though they do not by themselves confirm therapeutic efficacy or establish the fractions as antibiotic or antioxidant remedies. The differing chemical and biological profiles of the two fractions underscore how solvent choice affects both the quantity of phytochemicals extracted and the resulting bioactivity, a factor to be addressed in any effort to purify and characterise the responsible compounds. We recommend bioassay-guided fractionation to identify and isolate the most active compounds, compound-identification analysis (e.g. GC-MS/LC-MS) to determine which constituents are responsible for the observed activities, toxicity screening, and, if warranted by these results, subsequent *in vivo* studies. As the fractions' antimicrobial activity remains modest compared with standard antibiotics, testing them in combination with conventional antibiotics for potential synergy, an approach that has shown promise with other plant extracts, may offer an additional avenue for future work.

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