 DOI: https://doi.org/10.82351/wajp.vol37no2.484

Phytochemical Evaluation and Antimicrobial Activity of Selected Burundian Medicinal Plants Used in the Treatment of Skin Infections

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ABSTRACT

Background: Skin infections are among the most widespread healthcare threats in the tropics, including Burundi, and several plants have been used to treat skin infections as readily accessible alternative remedies. However, few studies have examined these plants to provide scientific evidence supporting their use in traditional medicine.

Objective: This study aimed to assess the antimicrobial properties of *Thunbergia petersiana* Lindau, *Clerodendrum schweinfurthii* Gürke, *Acalypha psilostachya* Hochst. ex A. Rich., and *Berkheya spekeana* Oliv. extracts against microorganisms implicated in skin infections.

Methods: The extract was prepared from the relevant plant parts, and phytochemical analysis was performed to determine the phytoconstituents. The agar well diffusion method was used to evaluate the antimicrobial activity of the plants against *Pseudomonas aeruginosa*, *Staphylococcus aureus, Candida albicans,* and *Trichophyton rubrum* and the broth dilution method was used to assess the minimum inhibitory concentrations (MICs).

Results: The phytochemical analysis confirmed the presence of various secondary metabolites, and *T. petersiana* showed the presence of all the phytochemicals. *C. schweinfurthii* and *T. petersiana* extracts displayed higher contents of flavonoids. *C. schweinfurthii, T. petersiana,* and *A. psilostachya* extracts demonstrated both antibacterial and antifungal properties, while *B. spekeana* was active only against fungi. *C. schweinfurthii* extract displayed a MIC of 7.81 µg/mL against *S. aureus* and 250 µg/mL against other organisms. *T. petersiana* extract exhibited a MIC of 250 µg/mL against tested microorganisms.

Conclusion: This study provided scientific evidence of the traditional uses of the plants in managing skin infections and revealed them as potential sources of alternative antimicrobial bioactive compounds.

Keywords: Burundi, Medicinal plants, Skin infections, Antimicrobial properties

 DOI: https://doi.org/10.82351/wajp.vol37no2.484

Évaluation phytochimique et activité antimicrobienne de certaines plantes médicinales burundaises utilisées dans le traitement des infections cutanées

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RÉSUMÉ

Contexte : Les infections cutanées figurent parmi les menaces sanitaires les plus répandues dans les régions tropicales, notamment au Burundi, et plusieurs plantes sont utilisées depuis longtemps comme remèdes alternatifs facilement accessibles pour traiter ces infections. Cependant, peu d'études ont examiné ces plantes afin de fournir des preuves scientifiques appuyant leur usage en médecine traditionnelle.

Objectif : Cette étude visait à évaluer les propriétés antimicrobiennes des extraits d'huile essentielle de *Thunbergia petersiana* Lindau, *Clerodendrum schweinfurthii* Gürke, *Acalypha psilostachya* Hochst. ex A. Rich., et *Berkheya spekeana* contre des micro-organismes impliqués dans les infections cutanées.

Méthodes : L'extrait a été préparé à partir des parties pertinentes de la plante, et une analyse phytochimique a été réalisée afin de déterminer les phytoconstituants. La méthode de diffusion en puits sur gélose a été utilisée pour évaluer l'activité antimicrobienne des plantes contre *Pseudomonas aeruginosa, Staphylococcus* aureus et *Candida*. albicans et *Trichophyton rubrum* et la méthode de dilution en bouillon a servi à déterminer les concentrations minimales inhibitrices (CMI).

Résultats : L'analyse phytochimique a confirmé la présence de divers métabolites secondaires et de T. petersiana a révélé la présence de l'ensemble des phytochimiques recherchés. Les extraits de C. schweinfurthii et de T. petersiana ont présenté des teneurs plus élevées en flavonoïdes. Les extraits de C. schweinfurthii, T. petersiana et A. psilostachya ont démontré des propriétés à la fois antibactériennes et antifongiques, tandis que B. spekeana n'était actif que contre les champignons. L'extrait de C. schweinfurthii a présenté une CMI de 7,81 ?g/mL contre S. aureus et de 250 ?g/mL contre les autres organismes. L'extrait de T. petersiana a montré une CMI de 250 ?g/mL contre les micro-organismes testés.

Conclusion : Cette étude a fourni des preuves scientifiques des usages traditionnels de ces plantes dans la prise en charge des infections cutanées et a révélé leur potentiel en tant que sources de composés bioactifs antimicrobiens alternatifs.

Mots-clés : Burundi, plantes médicinales, infections cutanées, propriétés antimicrobiennes

INTRODUCTION

Medicinal plants are the most potent sources of drugs to treat diseases and ensure well-being.[1] The knowledge of these plants is being passed from generation to generation, and they serve as a means of cure in nearly every society.[2] In the third world, the majority of people, i.e., 80 %, rely on herbal medicine for their well-being, and the level of use has similarly increased in the developed world.[3] Secondary metabolites from plants are expanding the drugs for the cure of diseases extensively.[4] Medicinal plants have a diverse group of phytochemicals, such as antibacterial, antiviral, and antifungal compounds.[5] Moreover, the abuse of antibiotics leading to antimicrobial resistance (AMR) has compelled the use of medicinal plants.[6] AMR is a threat to worldwide public health deserving the consideration of all government bodies and society.[7] Medicinal plants possess a large potential to serve as a goldmine of new antimicrobial agents with a high probability of closing the emerging gap in the remedy to the coming health risk of multi-drug resistance of microbial pathogens.[8] Interest in plants has promoted increased research and understanding of phytochemicals.[9] Various antibacterial activities of numerous plant metabolites with diverse active components have been reported.[10] Besides being readily accessible and affordable, phytochemicals or plant extracts are usually highly effective against infections and rarely have severe side effects.[11] They not only kill microbes but also influence cellular events in the pathological process, and pathogens are highly unlikely to acquire resistance to complex phytochemicals found in plant extracts.[12]

Skin infections and wounds can significantly affect individuals and may lead to mortality. The threat also arises from increasing antimicrobial resistance and the dissemination of resistant pathogenic agents in both healthcare facilities and the population.[13] *Pseudomonas aeruginosa, Acinetobacter spp.,* and *coagulase-negative staphylococci* such as *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* are pathogenic agents mostly responsible for skin infectious diseases.[14] The issue of bacterial skin infections caused by *Staphylococcus aureus*, for instance, is an alarming and unresolved challenge.[15] An ethnomedicinal survey of plants with antimicrobial properties indicated that skin infections are among the most prevalent infectious illnesses in Burundi, with diarrhoea and respiratory infections, particularly among children.[16] Their study also identified several plants utilised to manage these ailments, where, according to the survey, the majority of these herbal medicines are utilised to cure skin infectious diseases and diarrheal infections. However, documentation of Indigenous medicine in Burundi is seldom recorded,[17] leading to a lack of scientifically gathered or published data on medicinal plant species regarding their phytochemical profiles and potential biological effects.

Acalypha psilostachya, a perennial plant of the Euphorbiaceae family, is used in Burundi to manage different ailments, including wound healing among other traditional uses.[18] Up to 2008, the only known application of *A. psilostachya* was in Burundi,[19] and data bout this species are still scarce. *T. petersiana* is an erect or semi-prostrate perennial plant of the Acanthaceae family.[20] Similar to *Thunbergia alata*, it is used to manage various conditions, including microbial diseases.[16] *C. schweinfurthii* is a tropical dry-season-climbing liana or shrub of the Lamiaceae family. It occurs in seasonally dry tropical environments and is indigenous to tropical Africa.[21] It is also one of the most used plants in Burundi to manage infections.[16] *B. spekeana* is a sturdy, suffrutescent herb of the Asteraceae family, 2 m tall, branched, thorny, with white latex; stems and branches striate, pubescent, and leafy to the tip.[22] It is among the plants traditionally used to treat different diseases, including infectious ailments; classified among plants widely used in Burundi but unknown or little known outside Africa, not very well-known chemically and pharmacologically.[18] This study aimed to assess the antimicrobial activities of these underexplored plants against microorganisms implicated in skin infections, and conduct their phytochemical profiling.

MATERIALS AND METHODS

Test Organisms

This study targeted selected microorganisms implicated in skin infections: two bacteria, *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213); and two fungi, *Candida albicans* and *Trichophyton rubrum* (obtained at the Pharmaceutical Microbiology Laboratory, University of Ibadan).

Plants Materials

The aerial parts of *A. psilostachya* and *T. petersiana,* and the leaves of *C. schweinfurthii* were collected from the natural habitat in Nyabiraba, Burundi (Altitude: 1624 m, Latitude: 3°26 01.814''S, Longitude: 29°27'01.688''E), in January 2024. *B. spekeana* aerial parts were collected from Bukeye (Altitude: 2229 m, Latitude: 3°08'49,67''S, Longitude: 29°32'27,34''E), in the same period. The plants were identified and authenticated at the

Department of Biology, University of Burundi, and voucher numbers were assigned (*A. psilostachya*: NZP001, *T. petersiana*: NZP004, *B. spekeana*: NZP002, *C. schweinfurthii*: NZP003) and deposited in the herbarium of the University of Burundi. They were dried at room temperature and then pulverised into a coarse powder.

Extraction

Powdered plant (200 g) was macerated in 2,000 mL of 80% ethanol for 72 hours at room temperature, with occasional shaking. The extract was filtered using filter paper (Whatman filter paper No. 1) and concentrated using a rotary evaporator (Biobase, China, Model: BK-RE-1A) at 40^{o}C under reduced pressure. The extracts were kept in sterile, airtight containers at 4^{o}C in a refrigerator for further use.

Phytochemical Analysis

Preliminary phytochemical screening

The phytochemical screening of secondary metabolites including saponins, alkaloids, tannins, flavonoids, phenols, coumarins, quinones, cardiac glycosides, anthraquinones, and terpenoids was conducted following standard procedures.[23]

Quantitative phytochemical analysis

Alkaloids determination

Each plant sample, about 5 grams, was mixed with ethanol and 10 % acetic acid, covered, and left for four hours. The extract was washed and evaporated using a water bath (Gallenkamp, England). Concentrated ammonium hydroxide was gradually added. The solution was allowed to settle, and the residue was washed using diluted ammonium hydroxide and weighed.[24]

Determination of saponin content

Twenty grams of plant sample was dissolved in 20 % ethanol in a conical flask and heated in a water bath for 4 hours with constant agitation at about 55^{o}C. The solution was filtered, and the sediment was extracted with a further 20 % ethanol. The extracts were concentrated in a water bath at about 90^{o}C. The concentrate was transferred to a separatory funnel, and the diethyl ether was shaken vigorously with it. The aqueous component was retained. Purification was done once more. Sixty millilitres of n-butanol were added. The n-butanol mixed extracts were washed with 10 mL 5 % aqueous sodium chloride solution twice. The remaining solution was heated in a water bath. The samples were dried to constant weight after evaporation, and the saponin content was expressed as a percentage.[24]

Determination of total phenolics

Spectrophotometry was carried out for the determination of phenol content.[25] A solution of 1 mL of extract and 9 mL of distilled water was prepared in a 25 mL volumetric flask. One millilitre of Folin-Ciocalteu phenol reagent was added, and the mixture was shaken vigorously. After 5 minutes, 10 mL of 7 % sodium carbonate (Na_2CO_3) solution was added to the mixture, and the volume was adjusted to 25 mL. A series of Gallic acid standard solutions (20-100 µg/mL) was prepared. Following incubation at room temperature for 90 minutes, the absorbance was measured using a UV-visible spectrophotometer (Perkin Elmer, USA) at 750 nm. The phenol content was presented as milligrams of gallic acid equivalents per gram of extract (GAE/g).

Determination of tannin content

For each sample solution, 0.1 mL was placed in a volumetric flask with 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1 mL of 35 % Na_2CO_3 solution and diluted to 10 mL with distilled water. The solution was mixed and left to stand at room temperature for 30 minutes. The absorbance of the test and standard solutions (20, 40, 60, 80, and 100 µg/mL of Gallic acid) was recorded using a UV/Visible spectrophotometer at 725 nm. The tannin content was expressed in milligrams of gallic acid equivalents per gram of extract.[24]

Total flavonoids determination

One millilitre of the extract (1 mg/mL concentration) was combined with 4 mL of distilled water in a volumetric flask. 0.3 mL of 5 % sodium nitrite was added to the flask, and after 5 minutes, 0.3 mL of 10 % aluminium chloride was added. Two millilitres of 1 M sodium hydroxide were added, and the mixture was diluted to 10 mL with distilled water. The absorbance of test solutions and Quercetin standard solutions (20, 40, 60, 80, and 100 µg/mL) was read at 510 nm. The total flavonoid content was presented as milligrams of quercetin equivalents per gram of extract.[26]

Antimicrobial Susceptibility Testing

Agar well diffusion method

For each plant extract, 100 mg/mL, 50 mg/mL, and 25 mg/mL concentrations were used, and Dimethyl sulfoxide (DMSO) 10 % was utilised as a solvent. The microbial culture was spread onto Mueller-Hinton agar plates and Sabouraud dextrose agar plates using a non-toxic swab for bacteria (*Pseudomonas aeruginosa* (ATCC

27853) and *Staphylococcus aureus* (ATCC 29213)) and fungi (*Candida albicans* and *Trichophyton rubrum*), respectively. One hundred microliters were added to 6-mm-diameter wells using a micropipette under aseptic conditions. The plates were allowed to stand to allow pre-diffusion before incubation. The plates were incubated at 37°C for 24-48 h, after which the zones of inhibition were measured. Ciprofloxacin and ketoconazole were used as standard controls for bacteria and fungi, respectively.[27]

Minimum Inhibitory Concentration Determination

A stock solution of 1000 µg/mL was serially diluted in the microplate wells with Tryptone Soya Broth to achieve concentrations of 500 µg/mL, down to 3.91 µg/mL. Each organism was subcultured to prepare an overnight culture. Twenty microliters of the test microorganisms were added to each well with 100 µL of the extract solution. The microplate was incubated at 37°C for 24-48 hours. After that, 20 µL of 0.2 mg/mL P-Iodonitrotetrazolium violet (P-INT) was added to each well. After incubation for an additional 30 minutes, microbial growth was indicated by a transition from violet to red formazan colouration. A clear or yellow hue signified the absence of growth.[28]

Statistical Analysis

Microsoft Excel 365 was utilised to calculate the mean and standard deviation (SD) of the three replicates, and results were presented as Mean ± Standard deviation (SD).

RESULTS

Phytochemical Analysis

Phytochemical analysis revealed the presence of various secondary metabolites, saponins, alkaloids, flavonoids, tannins, phenols, coumarins, terpenoids, steroids, anthraquinones, quinones, and cardiac glycosides in the plant extracts (Table 1). *Thunbergia petersiana* extract was shown to contain all these phytochemicals, while *C. schweinfurthii* lacked steroids, and *A. psilostachya* and *B. spekeana* lacked anthraquinones. The quantitative phytochemical analysis of alkaloids, total phenol content, saponins, flavonoids, and tannins for all four plant extracts in Figure 1 showed that *C. schweinfurthii* had higher content in alkaloids (5.73 ± 0.11 %), phenols (197.96 ± 0.30 GAE/mg/g), and flavonoids (75.30 ± 0.30 QE/mg/g). In comparison, *T. petersiana* showed higher content in tannins (63.49 ± 0.40 GAE/mg/g) and saponins (4.37 ± 0.12 %).

Table 1: Phytochemical constituents of the plants

S/N	Phytochemicals	*A. psilostachya*	*B. spekeana*	*C. schweinfurthii*	*T. petersiana*
1	Saponins	+	+	+	+
2	Alkaloids	+	+	+	+
3	Flavonoids	+	+	+	+
4	Tannins	+	+	+	+
5	Phenols	+	+	+	+
6	Coumarins	+	+	+	+
7	Terpenoids	+	+	+	+
8	Steroids	+	+	-	+
9	Anthraquinones	-	-	+	+
10	Quinones	+	+	+	+
11	Cardiac glycosides	+	+	+	+

Key: *+ = Present*
- = Absent

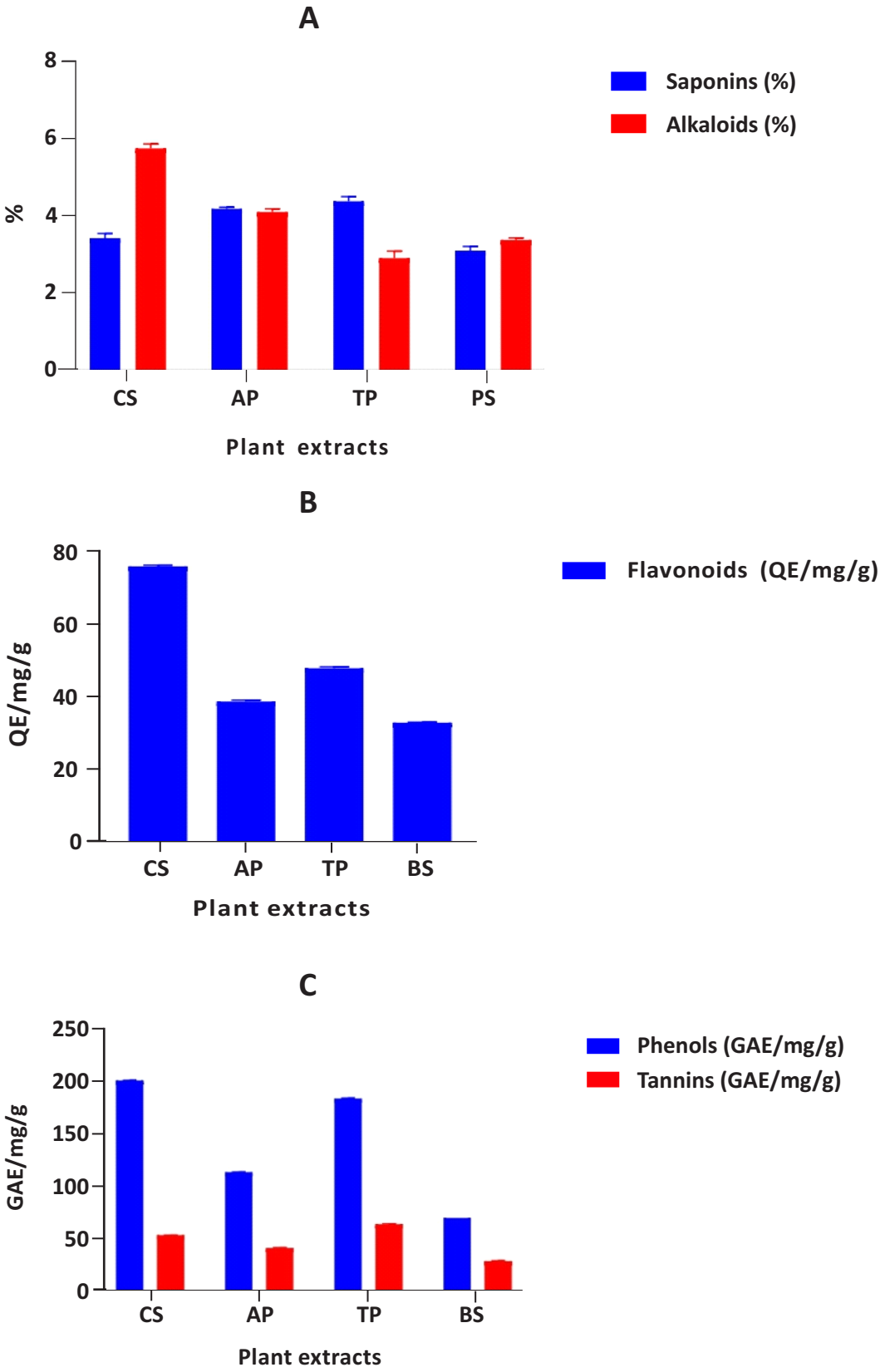


Figure 1: Quantitative phytochemical analysis, A: Tannin content (%), B: Total flavonoid content (QE/mg/g), C: Total saponin content and total phenol content (GAE/mg/g), CS: *Clerodendrum schweinfurthii*, AP: *Acalypha psilostachya*, TP: *Thunbergia petersiana*, BS: *Berkheya spekeana*

Antimicrobial Susceptibility Testing

The zones of inhibition of the plant extracts presented in Table 2 indicate that all the extracts exhibited antimicrobial properties. *C. schweinfurthii, T. petersiana,* and *A. psilostachya exhibited* both antibacterial and antifungal activities, while *B. spekeana* extract exhibited only antifungal activity. *Clerodendrum* schweinfurthii exhibited higher antibacterial potential, while *T. petersiana* extract exhibited higher antifungal activity. *Acalypha psilostachya* exhibited antibacterial and antifungal potential with small zones of inhibition. Fungi were not susceptible to ketoconazole (1 mg/mL) used as a positive control, which can be attributed to the low solubility of the drug in sterile water used as a vehicle.

Minimum Inhibitory Concentrations

The results of the broth dilution assay to determine the MICs of the two plant extracts with higher antimicrobial potential are presented in Table 3. *C. schweinfurthii* extract inhibited *Staphylococcus aureus* growth at a lower MIC (7.81 µg/mL), while the assay showed a higher MIC of 250 µg/mL against other pathogens.

Table 2: Antimicrobial activities of plant extracts using the agar well diffusion method

Organism	Concentration	Zones of inhibition (mm) of the plant extracts at different concentrations (Mean ± SD)					
	mg/mL	*C. schweinfurthii*	*T. petersiana*	*A. psilostachya*	*B. spekeana*	Cipro. (0.04mg/mL)	Keto. (1mg/mL)
S. aureus	100	19.66 ± 0.57	17.00 ± 1.00	9.33 ± 0.57	0.00 ± 0.00	29.33 ± 1.52	NA
	50	16.66 ± 0.57	13.66 ± 1.52	8.50 ± 0.71	0.00 ± 0.00		
	25	14.00 ± 0.57	10.66 ± 0.57	7.50 ± 0.71	0.00 ± 0.00		
P. aeruginosa	100	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28.66 ± 1.52	NA
	50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
	25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
C. albicans	100	18.66 ± 1.57	19.66 ± 1.53	18.00 ± 1.73	14.00 ± 1.73	NA	0.00 ± 0.00
	50	17.00 ± 1.00	17.00 ± 1.73	16.00 ± 1.00	13.00 ± 1.73		
	25	14.00 ± 1.00	15.00 ± 1.73	14.00 ± 1.73	11.66 ± 0.57		
T. rubrum	100	16.66 ± 1.15	19.33 ± 0.57	17.33 ± 1.15	14.33 ± 0.57	NA	0.00 ± 0.00
	50	14.00 ± 1.00	17.33 ± 0.57	15.33 ± 0.57	12.66 ± 0.57		
	25	11.66 ± 0.57	15.00 ± 1.00	12.33 ± 1.52	10.66 ± 0.57		

SD: standard deviation, NA: not applicable, Cipro: ciprofloxacin, Keto: ketoconazole.

Table 3: Minimum inhibitory concentrations (MIC) of the plants against the tested organisms

Microorganisms	*C. shweinfurthii* (µg/mL)	*T. petersiana* (µg/mL)	Ciprofloxacin (µg/mL)	Ketoconazole (µg/mL)
S. aureus	7.81	250.00	0.62	NA
P. aeruginosa	250.00	250.00	1.25	NA
C. albicans	250.00	250.00	NA	31.25
T. rubrum	250.00	250.00	NA	31.25

NA: not applicable

DISCUSSION

The four plants are sources of antimicrobial phytochemicals. Phenolic compounds are important secondary metabolites that act on many bacterial targets, including cytoplasmic membrane damage, topoisomerase inhibition, and NADH-reductase and ATP synthase inhibition.[29] Flavonoids exhibit antimicrobial properties through many mechanisms of action.[30] The analysis revealed the presence of terpenoids, which are phytochemicals abundant in nature and formed in response to microbial attacks. They exhibit enormous potential for growth inhibition against many bacteria through numerous modes of action such as membrane disruption, anti-quorum sensing, suppression of protein synthesis, and reducing Adenosine triphosphate availability.[31]

C. schweinfurthii and *T. petersiana exhibited* the strongest antimicrobial properties. Previous studies suggest that phenolic compounds are phytochemicals with significant antimicrobial properties. Our study indicated they were the most abundant compounds in the extracts from these two plants. Saponins, present in all plant extracts, have garnered considerable interest for their remarkable, broad-spectrum antibacterial activity.[32] They exhibit various bioactivities that make them useful in the pharmaceutical industry, including antibacterial, antiviral, and antiparasitic medicines.[33] In addition, alkaloids have influenced the production of various antibacterial medications.[34] They represent a possible new type of natural antibiotics with a broad antibacterial spectrum, rare side effects, and a low tendency to drug resistance.[35]

The strongest antibacterial potential of *C. shweinfurthii* aligns with its traditional uses. *C. shweinfurthii* is reported to be used against microbial infections in Burundi in a mono-herbal recipe, indicating traditional healers' claims that the herb could address these ailments effectively. Furthermore, this plant extract also displayed higher antifungal effects. *T. petersiana* has antibacterial properties, and the traditional claims indicate that the plant is effective in managing microbial infections, similarly to *Thunbergia alata*.[16] *Acalypha psilostachya* was shown to be a good candidate for microbial alternative treatment. The plant exhibited both antibacterial and antifungal properties against the microorganisms: *S. aureus*, *C. albicans*, and *T. rubrum*. As stated by Saddiqe and co-workers,[36] microorganisms can colonise infected body tissues and lead to various complications; the plant is used by traditional healers in wound healing, among many other documented traditional uses, in line with the uses of *Acalypha brachiata*, another species used in Burundi to manage skin infections.[16] On the other hand, *B. spekeana* could be a source of antifungal compounds. The plant was reported to be useful in the management of childhood diseases, among other uses, including fungal infections.[18]

CONCLUSION

This study provided scientific evidence supporting the traditional uses of the four Burudian plants. *Clerodendrum schweinfurthii, T. petersiana,* and *A. psilostachya* extracts showed both antibacterial and antifungal activities, while *B. spekeana* inhibited fungal growth. The phytochemical evaluation confirmed the presence of various secondary metabolites, including phytochemicals well known to exhibit antimicrobial properties. Further studies are crucial to assessing the antimicrobial activities of the plants against other pathogenic agents and isolating compounds responsible for these

properties.

ACKNOWLEDGMENTS

The African Union Commission provided funding for this research through the Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), Ibadan, Nigeria.

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