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PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL POTENTIAL OF *Campsis radicans* AND *Combretum indicum* EXTRACTS AGAINST UROPATHOGENIC BACTERIA ISOLATED FROM UTI PATIENTS

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ABSTRACT

This study investigated the solvent-dependent extraction efficiency, phytochemical composition, and antibacterial activity of leaf extracts from *Campsis radicans* and *Combretum indicum*. Leaf powders were subjected to Soxhlet extraction using hexane, methanol, and water. Extracts were concentrated, dried, and evaluated for percentage yield. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified using Folin–Ciocalteu and $AlCl_3$ colorimetric assays. Antibacterial efficacy was assessed by agar well diffusion against twenty clinical uropathogenic isolates, while Minimum Inhibitory Concentration (MIC) values were determined using the broth dilution method. Extraction yields varied with solvent polarity, ranging from 5.89–8.97% for *C. radicans* and 7.98–9.44% for *C. indicum*. Aqueous extracts exhibited the highest TPC (CRPAq: 766.84 ± 2.78 µg/g; CIPAq: 758.81 ± 4.81 µg/g), whereas methanolic extracts showed the greatest TFC (CRPMe: 473.94 ± 3.08 µg/g; CIPMe: 363.45 ± 1.18 µg/g). In antimicrobial analysis, methanolic extracts demonstrated superior activity (CRPMe: 9–12 mm; CIPMe: 11–14 mm), aqueous extracts showed moderate inhibition at higher concentrations, and hexane extracts were largely inactive. Ciprofloxacin (20–28 mm) served as a valid positive control. MIC results (0.78–6.25 mg/mL) revealed the strongest inhibition by CIPMe, particularly with MIC values as low as 0.78 mg/mL against several isolates. CRPMe displayed moderate but consistent activity. Overall, findings indicate that polar and semi-polar phytochemicals especially phenolics and flavonoids contribute significantly to the antibacterial potential of both species, with methanol being the most effective extraction solvent. *Combretum indicum* methanolic extract appears most promising for further purification and bioactive compound isolation.

Keywords **:** *Campsis radicans*, *Combretum indicum*, Soxhlet extraction, total phenolic content (TPC), total flavonoid content (TFC), antimicrobial activity, minimum inhibitory concentration (MIC), phytochemicals, methanolic extract, antibacterial assay.

Introduction

Medicinal plants remain one of the most valuable sources of therapeutic compounds and continue to play an essential role in traditional and modern healthcare systems. The increasing global burden of antimicrobial resistance (AMR) has intensified the search for novel, safe, and plant-derived antimicrobial agents. Plants are rich in diverse secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, saponins, and terpenoids which exhibit strong antimicrobial, antioxidant, and anti-inflammatory properties. Systematic extraction, phytochemical characterization, and antimicrobial evaluation of medicinal plants are therefore crucial for validating traditional claims and identifying new bioactive leads.

(A) **(B)**

Fig. 1. (A) *Campsis radicans*(Bignoniaceae) **(B)** *Combretum indicum* (Combretaceae).

Campsis radicans (family Bignoniaceae) and *Combretum indicum* (syn. *Quisqualis indica*, family Combretaceae) are two traditionally important medicinal plants extensively used in folk medicine across Asia. *C. radicans* is reported to possess anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties (Islam *et al.,* 2019; Mehmood *et al.,* 2022). Similarly, *C. indicum* has been widely used as a remedy for parasitic infections, fever, diarrhea, and microbial diseases, with several studies suggesting its richness in flavonoids, phenolics, triterpenoids, and alkaloids (Monica & Chaitanya, 2023; Ramtin *et al.*, 2022). Despite their traditional significance, comprehensive comparative studies examining solvent-dependent extraction efficiency, phytochemical composition, and antibacterial activity of these two species remain limited. Solvent polarity plays a critical role in the extraction of plant metabolites, influencing both yield and the spectrum of extracted compounds. Techniques such as Soxhlet extraction efficiently isolate thermally stable phytochemicals, while quantitative assays like the Folin–Ciocalteu and $AlCl_3$ colorimetric methods provide reliable estimations of total phenolic content (TPC) and total flavonoid content (TFC). Antibacterial assays, including agar well diffusion and minimum inhibitory concentration (MIC) determination, further allow evaluation of the therapeutic potential of plant extracts.

The present study investigates the extraction efficiency, phytochemical composition, and antibacterial activity of methanolic, aqueous, and hexane extracts of *Campsis radicans* and *Combretum indicum*. Using clinical bacterial isolates obtained from urine samples of suspected UTI patients, the study aims to compare solvent-dependent variations in phenolic/flavonoid levels and antimicrobial potency. The findings contribute to the identification of promising plant-based antimicrobial agents and provide a scientific foundation for future bioassay-guided isolation of active compounds.

Materials and Methods

Materials

Analytical-grade chemicals and microbiological reagents were employed throughout the study. Plant extraction was performed using hexane (Qualigens), methanol (SDFCL), and distilled water as solvents. Filtration was carried out using Filter Star filter papers, and all glassware including beakers, conical flasks, round-bottom flasks, volumetric flasks, measuring cylinders, pipettes, funnels, reagent bottles, and glass rods was sourced from Borosil. Reagents used for phytochemical assays included Folin Ciocalteu reagent, sodium carbonate, aluminum chloride ($AlCl_3$), potassium acetate, and gallic acid and quercetin standards. Antimicrobial susceptibility testing utilized Mueller–Hinton Agar (MHA, HiMedia), ciprofloxacin discs (positive control), sterile cotton swabs, micropipettes with sterile tips, and 0.22 µm syringe filters, with respective solvents serving as negative controls.

Plant material was extracted using a Borosil Soxhlet apparatus connected to a heating mantle and Borosil round-bottom extraction flasks, followed by concentration of the extracts using a rotary evaporator. The analytical instrumentation comprised a UV–Visible spectrophotometer (Systronics), water bath (iTherm AI-778), incubator, autoclave, pH meter, analytical balance, laminar airflow cabinet, and a digital caliper for measuring inhibition zones.

Collection and Preparation of Plant Material

Fresh, healthy leaves of *Campsis radicans* and *Combretum indicum* were collected from nurseries and local regions of Allahabad, Uttar Pradesh, India. Plant species were authenticated by experts from the Department of Botany, SHUATS.

Leaves were washed thoroughly with distilled water to remove dust and debris and shade-dried for approximately 15 days. Dried leaves were powdered using a mechanical grinder and stored in airtight containers until extraction.

Extraction of Plant Material

A total of 30g of powdered leaves from each plant species (*Campsis radicans* and *Combretum indicum*) was used for solvent extraction. Soxhlet extraction was performed separately with three solvents of differing polarity:

- **Hexane** (non-polar)
- **Methanol** (polar protic)
- **Distilled water** (highly polar)

For each extraction cycle, 200 mL of the respective solvent was added to a round-bottom flask connected to the Soxhlet extractor. Horwitz (2010). The extraction was allowed to run for 6–8 hours, or until the siphon solvent in the apparatus became clear, indicating complete extraction of soluble phytochemicals.

Following completion of each extraction, the solvent fractions were concentrated using a rotary evaporator under reduced pressure at controlled temperatures:

- **Hexane extracts:** 40–45°C
- **Methanol extracts:** 45–50°C
- **Aqueous extracts:** 55–60°C

The resulting semi-solid extracts were air-dried to remove residual solvent after which they were accurately weighed. Each extract was labelled according to plant species and solvent type and stored in amber vials at 4°C until further phytochemical and antimicrobial analyses.

Determination of Extraction Yield

The extraction yield of *Campsis radicans* and *Combretum indicum* leaf extracts was determined to evaluate the efficiency of different solvents in extracting phytoconstituents. After solvent evaporation and air-drying of the filtrates, the dried crude extracts obtained from each solvent system were accurately weighed. The percentage yield was then calculated using the standard formula:

$$\text{Yield}\,(\%) = \frac{\text{Weight of dried extract}}{\text{Weight of leaf powder}} \times 100$$

This calculation provided a quantitative estimate of the amount of bioactive material extracted relative to the initial plant mass and enabled a comparative assessment of solvent efficiency. Differences in extraction yield reflect the varying polarity and solubility characteristics of phytochemicals present in the plant material, with polar solvents such as methanol typically yielding higher extractable content than non-polar solvents like hexane. The extraction yield values served as an important indicator of solvent performance and guided the selection of extracts for subsequent phytochemical and antimicrobial analyses. Similar methodological approaches have been recommended in phytochemical research for evaluating extraction efficiency (Harborne, 1998; Trease & Evans, 2002).

The methanolic extract of *Campsis radicans* was denoted as CRPMe, the aqueous extract as CRPAq, and the hexane extract as CRPHe.

Phytochemical Screening

Determination of Total Phenolic Content (TPC)

The extracts were prepared in ethanol at a concentration of 1 mg/mL. For the TPC test, 0.1 mL of each extract (CRPMe, CRPAq, CRPHe, CIPMe, CIPAq, CIPHe) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent, followed by 2.0 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark at room temperature for 1.5 hours (Singleton & Rossi 1965). After incubation, absorbance was recorded at 765 nm using a UV–Vis Double Beam Spectrophotometer (Systronic). Gallic acid standards (10–100 µg/mL) were prepared and treated in the same way. The total phenolic content was calculated from the gallic acid calibration curve and expressed as mg Gallic Acid Equivalent (GAE) per gram of extract. Hossain *et al.* (2017).

Determination of Total Flavonoid Content (TFC)

The extracts were prepared in ethanol at a concentration of 1 mg/mL. For TFC estimation, 500 µL of each extract (CRPMe, CRPAq, CRPHe, CIPMe, CIPAq, CIPHe) was mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. After incubation, absorbance was recorded at 415 nm using a UV–Vis Double Beam Spectrophotometer (Systronic). Standard quercetin solutions (10–100 µg/mL) were prepared and treated in the same way. The Total Flavonoid Content was calculated from the quercetin calibration curve and expressed as mg Quercetin Equivalent (mg QE) per gram of extract. Hossain *et al.* (2017).

Antibacterial Activity Against Test Organisms

The antimicrobial activity of the hexane, methanolic, and aqueous extracts of *Campsis radicans* and *Combretum indicum* (CRPMe, CRPHe, CRPAq, CIPMe, CIPHe, CIPAq) was evaluated using the agar well diffusion method. Mueller–Hinton Agar (MHA) was prepared according to HiMedia specifications for all bacterial assays. The media were dissolved in distilled water, sterilized at 121°C and 15 psi for 15 minutes in an autoclave, and aseptically poured (30 mL per plate) into sterile borosilicate Petri dishes inside a laminar airflow cabinet Wiegand *et al.* (2008).

Bacterial suspensions were standardized to 0.5 McFarland turbidity (~1.5×10^8 CFU/mL). Each plant extract was dissolved in its respective solvent (methanol, distilled water, or hexane), filtered through 0.22 µm syringe filters, and prepared at three concentrations: 100 mg/mL (C1), 50 mg/mL (C2), and 25 mg/mL (C3). The agar plates were uniformly inoculated using sterile cotton swabs to ensure a confluent lawn of bacterial growth. After allowing the inoculum to dry for approximately 10 minutes, 8 mm diameter wells were made using a sterile cork borer. Each well was filled with 50 µL of the prepared extract. Ciprofloxacin (100 ppm) served as the positive control, and solvent blanks served as negative controls. All inoculated plates were sealed with parafilm and incubated at 37°C for 24 hours. After incubation, plates were examined for the presence of clear inhibition zones around the wells. The diameter of each zone of inhibition was measured in millimetres using a digital Vernier caliper. All experiments were conducted in triplicate to ensure accuracy and reproducibility.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the plant extracts was determined using the broth dilution method. Nutrient broth was prepared according to standard composition and sterilized by autoclaving. For each extract, a series of ten test tubes was arranged, beginning with the highest test concentration (25%) and followed by successive lower concentrations. Sterility control (nutrient broth only), growth control (broth with bacterial inoculum but without extract), and a positive control (broth with 0.1 mg/mL ciprofloxacin) were included in each set. After preparation, 50 µL of a 24-hour-old bacterial inoculum was added to each test and growth control tube, while the positive control tube received 15 µL of ciprofloxacin. All tubes were plugged and incubated at 37°C (Humphries *et al.*, 2021). Following incubation, tubes were visually examined for turbidity as evidence of microbial growth. Optical density (OD) readings of each tube were then recorded at 630 nm using a UV-Visible Double Beam Spectrophotometer (PC-Based Double Beam UV-VIS Spectrophotometer 2201). The MIC value was defined as the lowest extract concentration that showed no visible turbidity, indicating complete inhibition of bacterial growth.

Method (Statistical Analysis)

Quantitative data obtained from the antimicrobial activity assay (zone of inhibition measurements) were analyzed using one-way analysis of variance (ANOVA) to compare mean antibacterial responses among six treatment groups (A–F) (Shaw *et al*., 2014). The analysis assumed a normal (Gaussian) distribution of data. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using GraphPad Prism software, and results were expressed as mean ± standard deviation.

Result

Extraction Yield

The extraction yield of *Campsis radicans* and *Combretum indicum* leaf powders obtained using methanolic, aqueous, and hexane solvents is summarized in Table 1. A total of six samples coded CRPMe, CRPAq, CRPHe, CIPMe, CIPAq, and CIPHe were analyzed. Among *Campsis radicans* samples, the highest extraction yield was recorded for the hexane extract (CRPHe), which produced 8.97%, followed by the methanolic extract (CRPMe) with 7.52%, while the aqueous extract (CRPAq) gave 5.89%.

For *Combretum indicum*, the highest yield was obtained from the aqueous extract (CIPAq), yielding 8.76%, followed closely by the methanolic extract (CIPMe) with 7.98%, while the hexane extract (CIPHe) produced the lowest yield of 9.44%.

Table 1 : Yield obtained from samples coded (CRPMe, CRPAq, CRPHe, CIPMe, CIPAq & CIPHe) after extraction.

S. No	Sample Code	Yield content (%)
1	CRPMe	7.52
2	CRPAq	5.89
3	CRPHe	8.97
4	CIPMe	7.98
5	CIPAq	8.76
6	CIPHe	9.44

The results presented in Table 1 show clear solvent-dependent variations in extraction efficiency for both plant species. In *Campsis radicans*, hexane yielded the highest extractable content, indicating the presence of notable non-polar phytoconstituents. In

contrast, *Combretum indicum* exhibited comparatively higher yields in aqueous and methanolic extracts, suggesting a greater abundance of polar or water-soluble metabolites. These differences reflect species-specific phytochemical composition and demonstrate the influence of solvent polarity on extraction performance.

Phytochemical Screening

Total Phenolic Content (TPC)

The Total Phenolic Content of the six extracts showed distinct variation depending on the plant species and solvent used. The highest TPC value was recorded in the aqueous extract of *Campsis radicans* (CRPAq: 766.84 ± 2.78 µg/g), followed closely by the aqueous extract of *Combretum indicum* (CIPAq: 758.81 ± 4.81 µg/g). Among the methanolic extracts, *C. indicum* (CIPMe) exhibited a higher phenolic content (694.61 ± 7.35 µg/g) than *C. radicans* (CRPMe: 609.55 ± 4.81 µg/g). Hexane extracts contained the lowest phenolic levels, with CRPHe at 579.06 ± 5.56 µg/g and CIPHe at 514.86 ± 7.35 µg/g. These findings demonstrate significant solvent-dependent differences in phenolic extraction efficiency among samples.

Table 2 : Observed Total Phenolic Content of sample coded CRPMe, CRPAq, CRPHe, CIPMe, CIPAq, CIPHe.

S. No	Test	Sample code	Observation
1	Total Phenolic Content (µg/g)	CRPMe	609.55 ± 4.81
2		CRPAq	766.84 ± 2.78
3		CRPHe	579.06 ± 5.56
4		CIPMe	694.61 ± 7.35
5		CIPAq	758.81 ± 4.81
6		CIPHe	514.86 ± 7.35

As summarized in Table 2, aqueous extracts of both *C. radicans* and *C. indicum* exhibited the highest phenolic content, confirming that phenolic compounds in these species are predominantly water-soluble. Methanolic extracts also contained substantial phenolic levels, whereas hexane extracts showed the lowest values, consistent with the non-polar nature of this solvent. These results highlight the selective extraction capabilities of different solvents and affirm the strong association between solvent polarity and phenolic recovery.

Total Flavonoid Content (TFC)

Quantification of Total Flavonoid Content revealed that methanolic extracts contained the highest levels of flavonoids across both plant species. The highest TFC value was observed in *Campsis radicans* methanolic extract (CRPMe: 473.94 ± 3.08 µg/g), followed by *Combretum indicum* methanolic extract (CIPMe: 363.45 ± 1.18 µg/g). Aqueous extracts exhibited moderate flavonoid levels (CIPAq: 262.56 ± 1.18 µg/g; CRPAq: 214.18 ± 2.37 µg/g), whereas hexane extracts recorded the lowest TFC values, with CIPHe at 189.48 ± 3.30 µg/g and CRPHe at 171.29 ± 1.78 µg/g**.**

Table 3 : Observed Total Flavonoid Content of sample coded CRPMe, CRPAq, CRPHe, CIPMe, CIPAq, CIPHe.

S. No	Test	Sample code	Observation
1	Total Flavonoid Content (µg/g)	CRPMe	473.94 ± 3.08
2		CRPAq	214.18 ± 2.37
3		CRPHe	171.29 ± 1.78
4		CIPMe	363.45 ± 1.18
5		CIPAq	262.56 ± 1.18
6		CIPHe	189.48 ± 3.30

Table 3 shows that methanolic extracts of both plants possessed the highest flavonoid concentrations, indicating that flavonoids in these species are mainly polar to semi-polar in nature. Aqueous fractions exhibited moderate levels, while hexane extracts contained minimal flavonoids, reflecting their limited solubility in non-polar solvents. Overall, the results suggest that methanol is the most efficient solvent for extracting flavonoid constituents from both *C. radicans* and *C. indicum*.

Antimicrobial Activity

The antimicrobial evaluation of plant extracts CRPMe, CRPHe, CRPAq, CIPMe, CIPHe, and CIPAq against twenty clinical bacterial isolates (BA-34 to Mac-176) revealed marked variations in activity depending on plant species, extraction solvent, and extract concentration. Overall, methanolic extracts exhibited the strongest antibacterial activity, with CIPMe (*Combretum indicum*) producing inhibition zones ranging from 11 to 14 mm against most tested isolates, followed by CRPMe (*Campsis radicans*), which showed moderate but consistent activity with inhibition zones of 9–12 mm. Aqueous extracts displayed moderate antibacterial effects only at the highest concentration (100 mg/mL), producing inhibition zones between 6 and 15.5 mm against selected strains such as BA-34, BA-57, BA-60, BA-70, and BA-83. In contrast, hexane extracts showed minimal or no antibacterial activity; CRPHe produced weak inhibition (7–8.5 mm) only at 100 mg/mL, while CIPHe exhibited almost no inhibitory effect against any isolate. For several organisms, including BA-96, BA-130, BA-142, BA-174, BA-214, BA-302, BA-382, and multiple MacConkey agar isolates, hexane extracts showed no detectable antibacterial action at any tested

concentration. Ciprofloxacin (100 ppm), used as a positive control, consistently produced the largest inhibition zones (20–28 mm), validating the assay, whereas all negative controls (methanol, aqueous, and hexane solvents) showed no inhibition. Collectively, these findings indicate that methanol is the most effective solvent for extracting antibacterial compounds, followed by water, while non-polar hexane extracts are largely ineffective, establishing CIPMe as the most potent extract and CRPMe as a moderately active but reliable antibacterial agent. For clarity and consistency, blood agar plates were abbreviated as BA and MacConkey agar plates as MCA throughout the study.

Table 4 : Antibacterial activity of different plant extracts against clinical bacterial isolates showing activity levels, and inhibition zone ranges.

S. No.	Extract Code	Activity Level	Zone of Inhibition Range (mm)
1	CIPMe	High	11–14
2	CRPMe	Moderate–High	9–12
3	CIPAq	Moderate	6–15.5
4	CRPAq	Moderate	6–15.5
5	CRPHe	Weak	7–8.5
6	CIPHe	No/Negligible Activity	0–6
7	Positive Control	Very High	20–28
8	Negative Controls	Inactive	0

The comparative data presented in Table 4 further substantiate the trends observed in the antimicrobial screening. Methanolic extracts, particularly CIPMe, consistently demonstrated the highest inhibitory performance, whereas aqueous extracts displayed only moderate activity and hexane extracts showed minimal to no inhibition. The clear gradient in activity from very high inhibition by the positive control to complete inactivity of the solvent controls confirms the reliability of the assay. Overall, the results highlight the superior efficiency of methanol in extracting antibacterial constituents from both plant species and provide a strong basis for prioritizing methanolic fractions in subsequent purification and bioactivity-guided studies.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) values obtained for the plant extracts showed substantial variation in antibacterial potency across the tested microorganisms. The methanolic extract of *Combretum indicum* (CIPMe) demonstrated the strongest inhibitory activity, with the lowest MIC value of 0.78 mg/mL recorded against BA-130(A), BA-174, BA-302(A), and CRPAq also showed a low MIC of 0.78 mg/mL against BA70(A). CIPMe additionally exhibited moderate inhibition (1.56–3.13 mg/mL) against BA-142, BA-214, BA70(A), Mac-102, Mac-S2(B), Mac-S40 and Mac-S99. The methanolic extract of *Campsis radicans* (CRPMe) displayed activity across all isolates, with MIC values generally falling between 3.13–6.25 mg/mL, except for BA-174 which showed higher sensitivity (1.56 mg/mL). Aqueous extracts revealed selective activity, while hexane extracts exhibited minimal or no inhibitory effect. Optical density readings at 630 nm confirmed inhibition patterns, with tubes showing low OD values (0.02–0.09) corresponding to complete inhibition. The overall MIC range recorded for all extracts was 0.78–6.25 mg/mL, with methanolic extracts consistently producing the lowest inhibitory concentrations.

Table 5 : Minimum Inhibitory Concentration (MIC) values of plant extracts (CRPMe, CIPMe, CRPAq, CRPHe, CIPAq) against selected clinical bacterial isolates.

S.No.	Code	Microorganism	MIC (mg/ml)
1	CRPMe	BA 34	3.13
2	CIPMe	BA 34	6.25
3	CIPMe	BA-130-(A)	0.78
4	CRPMe	BA-130-(A)	3.13
5	CIPMe	BA-142	1.56
6	CRPMe	BA-142	3.13
7	CIPMe	BA-174	0.78
8	CRPMe	BA-174	1.56
9	CIPMe	BA-184	3.13
10	CRPMe	BA-184	3.13
11	CIPMe	BA-214	1.56
12	CRPMe	BA-214	6.25
13	CIPMe	BA-302-(A)	0.78
14	CRPMe	BA-302-(A)	3.13
15	CIPMe	BA-382	3.13
16	CRPMe	BA-382	3.13
17	CRPMe	BA70-(A)	3.13
18	CIPMe	BA70-(A)	1.56
19	CRPAq	BA70-(A)	0.78
20	CIPMe	BA96	6.25
21	CRPMe	BA96	3.13
22	CIPMe	MAC-102	1.56
23	CRPMe	MAC-102	3.13
24	CIPMe	MAC-176	6.25
25	CRPMe	MAC-176	6.25
26	CIPMe	MAC-S1-(B)	6.25
27	CRPMe	MAC-S1-(B)	3.13
28	CIPMe	MAC-S2-(B)	1.56
29	CRPMe	MAC-S2-(B)	6.25
30	CIPMe	MAC-S20	3.13
31	CRPMe	MAC-S20	6.25
32	CIPMe	MAC-S40	1.56
33	CRPMe	MAC-S40	6.25

34	CIPMe	MAC-S99	3.13
35	CRPMe	MAC-S99	3.13

The detailed MIC values for all extracts against the tested microorganisms are summarized in Table 5. The results clearly show that different bacterial isolates exhibited varying levels of susceptibility toward the plant extracts. Methanolic extracts demonstrated the strongest inhibitory effect, particularly CIPMe, which showed the lowest MIC values across multiple strains. In contrast, aqueous extracts showed selective inhibition, while hexane extracts exhibited minimal or no activity. These findings indicate that solvent polarity greatly influences the antibacterial potency of plant extracts.

Statistical Analysis

One-way analysis of variance (ANOVA) revealed a highly significant difference in antimicrobial activity among the six treatment groups (A–F), as reflected by variations in the mean zones of inhibition ($F(5, 114) = 81.64$, $P < 0.0001$). The model accounted for a substantial proportion of the total variability in antibacterial response ($R^2 = 0.7817$). Tests for homogeneity of variances indicated significant heteroscedasticity, as confirmed by the Brown–Forsythe test ($F(5, 114) = 2.794$, $P = 0.0203$) and Bartlett's test ($\chi^2 = 94.87$, $P < 0.0001$). Despite unequal variances, the ANOVA results demonstrated robust and statistically supported differences in antimicrobial efficacy among the tested treatments based on 120 observations across six groups.

Discussion

Extraction Yield

The extraction yield showed noticeable variation across solvents, indicating the important role of solvent polarity in phytochemical extraction. In *Campsis radicans*, the highest yield was obtained using hexane (CRPHe: 8.97%), suggesting the presence of a moderate amount of non-polar constituents such as lipids, waxes, and hydrophobic secondary metabolites. The methanolic extract (CRPMe: 7.52%) produced a slightly lower yield, reflecting methanol's efficiency in extracting both polar and semi-polar phytochemicals. The aqueous extract (CRPAq: 5.89%) showed the lowest yield among the three, indicating relatively fewer water-soluble compounds in this species.

In *Combretum indicum*, a different extraction pattern was observed. The aqueous extract (CIPAq: 8.76%) exhibited the highest yield, suggesting a higher proportion of water-soluble constituents such as carbohydrates, glycosides, and certain phenolics. Methanol also extracted a considerable amount (CIPMe: 7.98%), indicating the presence of polar and semi-polar metabolites. Interestingly, the hexane extract (CIPHe: 9.44%) also produced a relatively high yield, implying that *C. indicum* contains an appreciable amount of non-polar compounds as well.

Overall, the results reveal that no single solvent was uniformly superior for both species. Instead, the extraction behavior varied according to plant chemistry. Methanol and aqueous solvents were effective for *C. indicum*, whereas hexane yielded the highest extractability for *Campsis radicans*. Such solvent-dependent variations highlight the importance of selecting extraction systems based on plant-specific phytochemical profiles and research objectives.

Total Phenolic Content (TPC)

The TPC results clearly indicate that aqueous solvents were the most efficient in extracting phenolic compounds from both *Campsis radicans* and *Combretum indicum*. This is consistent with the hydrophilic nature of most phenolics, including tannins, phenolic acids, and some glycosylated polyphenols, which readily dissolve in water. Methanol also produced high phenolic yields, reflecting its strong ability to extract both polar and moderately polar compounds. Hexane yielded the lowest phenolic content, confirming that phenolics have minimal solubility in non-polar solvents.

Overall, the results support that both plant species are rich in phenolic constituents, with solvent polarity playing a decisive role in extraction efficiency. The findings also align with previous studies showing higher phenolic recovery in polar solvents such as water and methanol.

Total Flavonoid Content (TFC)

Flavonoid content was highest in methanolic extracts, demonstrating that methanol is the most suitable solvent for extracting these polyphenolic compounds due to its intermediate polarity and ability to solubilize both aglycones and glycosylated forms. Aqueous extracts contained lower flavonoid levels, likely due to the limited solubility of many flavonoids in water. Hexane, being non-polar, showed minimal flavonoid extraction, confirming that flavonoids rich in hydroxyl groups are largely polar and poorly soluble in non-polar solvents. These findings highlight that both *Campsis radicans* and *Combretum indicum* possess significant quantities of flavonoids, which contribute to their antioxidant and therapeutic properties. The results also align with established literature suggesting that alcohols, particularly methanol, maximize flavonoid extraction efficiency.

Antimicrobial Activity

The antimicrobial results demonstrate that solvent polarity plays a decisive role in extracting bioactive compounds responsible for microbial inhibition in *Campsis radicans* and *Combretum indicum*. Methanolic extracts, particularly CIPMe, exhibited the highest antibacterial activity across all tested isolates, indicating that the most potent antimicrobial compounds in these plants are polar to semi-polar in nature and therefore readily soluble in methanol. CRPMe also showed moderate but consistent inhibition, supporting the presence of methanol-soluble antimicrobial phytochemicals in *Campsis radicans*. In contrast, aqueous extracts produced only mild activity at the highest concentration, which suggests that although some polar antimicrobial compounds exist, their concentrations may be lower or less potent than those extracted by methanol. Hexane extracts displayed little to no inhibitory effect, confirming that non-polar constituents in both plants possess limited antibacterial potential. The stronger antibacterial performance of *Combretum indicum* methanolic extract compared to *Campsis radicans* further highlights species-specific phytochemical differences. Moreover, the clear concentration-dependent increase in inhibition zones supports dose-responsive antimicrobial behavior. The overall trend methanol > aqueous > hexane aligns with established knowledge that phenolics, flavonoids, tannins, and other antimicrobial secondary metabolites are predominantly polar compounds, efficiently extracted using alcohols. The comparison with ciprofloxacin also suggests that while plant extracts possess notable antibacterial activity, they are less potent than commercial antibiotics, yet still demonstrate promising potential for natural antimicrobial agent development.

Minimum Inhibitory Concentration(MIC)

The MIC findings clearly indicate that the antimicrobial activity of *Campsis radicans* and *Combretum indicum* is strongly dependent on both solvent polarity and phytochemical solubility. Methanol-extracted samples, particularly CIPMe, were the most effective, demonstrating significantly lower MIC values, which highlights the presence of potent polar and semi-polar antimicrobial compounds such as flavonoids, phenolics, and alkaloids. CRPMe also showed consistent activity, suggesting that *C. radicans* possesses antimicrobial metabolites, though in lower concentrations compared to *C. indicum*. In contrast, the limited effectiveness of aqueous extracts and the poor activity of hexane extracts confirm the low solubility of major antimicrobial phytochemicals in non-polar solvents. The strong correlation between low MIC values and extracts rich in phenolics/flavonoids is in agreement with established literature describing these compounds as key contributors to bacterial growth inhibition. Overall, the MIC results reinforce that *Combretum indicum* methanolic extract possesses the highest antimicrobial potential among all tested samples and warrants further purification and characterization to identify its active constituents.

Statistical Analysis

The one-way ANOVA results demonstrated highly significant differences among the six treatment groups, indicating strong treatment-dependent effects on the measured response. The high R^2 value suggests that most of the observed variability was explained by treatment differences. Although variance homogeneity was violated, such heterogeneity is common in biological data, and the robust F statistic supports the reliability of the findings. Overall, the analysis confirms meaningful and statistically supported differences among treatments.

Conclusion

The present study provides a comprehensive evaluation of the extraction efficiency, phytochemical composition, and antibacterial potential of *Campsis radicans* and *Combretum indicum* leaf extracts using solvents of varying polarity. The findings collectively highlight the strong influence of solvent selection on extract yield, phytochemical recovery, and antimicrobial performance, emphasizing the importance of optimizing extraction conditions for medicinal plant research. Extraction yields varied noticeably between species and solvents, with *C. radicans* showing maximum yield in hexane (8.97%), while *C. indicum* exhibited the highest yield in aqueous and hexane fractions (8.76% and 9.44%, respectively). These results reflect inherent differences in the chemical composition of each species and illustrate the solvent-specific solubility of phytoconstituents ranging from non-polar lipids to polar phenolic compounds.

Quantitative phytochemical analysis revealed significant variations in TPC and TFC across extracts. Aqueous extracts were rich in phenolic constituents, demonstrated by high TPC values in CRPAq (766.84 ± 2.78 µg/g) and CIPAq (758.81 ± 4.81 µg/g), consistent with the hydrophilic nature of most phenolic compounds. In contrast, methanolic extracts possessed the highest flavonoid concentrations, indicating that methanol is particularly efficient at extracting semi-polar flavonoids, which substantially contribute to antioxidant and antimicrobial functions. Hexane extracts consistently showed low TPC and TFC values,

highlighting their limited ability to solubilize phenolics and flavonoids.

The antimicrobial activity assessment further established that phytochemical richness is closely associated with antibacterial potency. Methanolic extracts, particularly CIPMe from *C. indicum*, demonstrated the strongest inhibitory effects, producing zones of inhibition between 11–14 mm and exhibiting MIC values as low as 0.78 mg/mL against several clinical isolates. CRPMe from *C. radicans* also showed moderate antibacterial action, indicating the presence of bioactive compounds, although in lower concentrations than *C. indicum*. Aqueous extracts displayed selective activity only at higher concentrations, while hexane extracts were largely inactive, correlating with their low phytochemical content. The MIC results mirrored these trends, reaffirming methanolic extracts as the most effective antimicrobials, followed by aqueous extracts, with hexane extracts showing negligible activity.

Overall, the study demonstrates that both *Campsis radicans* and *Combretum indicum* possess significant polar and semi-polar bioactive constituents responsible for their antibacterial properties. Methanol emerged as the most efficient solvent for extracting biologically active phytochemicals, particularly phenolics and flavonoids, which likely drive the inhibitory effects observed. Among the tested extracts, methanolic *Combretum indicum* (CIPMe) consistently exhibited the highest antimicrobial potency, making it a strong candidate for advanced pharmacological investigation. These findings lay a solid foundation for future studies focusing on bioassay-guided fractionation, isolation, and structural identification of active compounds. Further toxicity, mechanistic, and in vivo evaluations are recommended to establish the therapeutic potential and clinical applicability of these plant-derived antimicrobial agents.

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Competing Interests

The authors declare that they have no known financial or non-financial competing interests that could have appeared to influence the work reported in this paper.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Ethics Committee prior to the collection and analysis of human urine samples.

Consent to Participate

Written informed consent was obtained from all participants or their legal guardians before inclusion in the study.

Authors' Contributions

The first author conducted the experimental work, data collection, analysis, and manuscript preparation. The supervisor provided guidance in study design, data interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

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