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Bioactivity of *Combretum aculeatum* Extracts against Methicillin-Resistant *Staphylococcus aureus* (MRSA) Clinical Isolates: Phytochemical and Antioxidant Insights

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ABSTRACT

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major challenge to the treatment of skin and soft tissue infections, particularly in resource-limited countries. Medicinal plants represent a valuable source of alternative antimicrobial agents. This study investigated the bioactivity of ethanolic and ethyl acetate extracts of *Combretum aculeatum* leaves, focusing on their phytochemical composition, antioxidant potential, and antibacterial activity against clinical MRSA isolates. Qualitative phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, alkaloids, saponosides, quinonic derivatives, mucilage, and reducing compounds. Quantitative analyses showed comparable total phenolic and condensed tannin contents between extracts, while flavonoids and hydrolysable tannins were significantly higher in the ethanolic extract. Antioxidant activity assessed by DPPH radical scavenging and phosphomolybdenum reduction assays demonstrated substantial and comparable reducing capacities for both extracts. Antibacterial evaluation using the agar disk diffusion method showed that both extracts inhibited the growth of fifty-four clinical MRSA isolates from wounds, pus, abscesses, and boils. Principal component analysis highlighted relationships between strain susceptibility, extract type, and clinical origin. Overall, *C. aculeatum* leaf

extracts exhibit significant antioxidant and anti-MRSA activities, supporting their traditional use and their potential as sources of bioactive compounds for managing resistant infections.

1. INTRODUCTION

The development of antimicrobial resistance continues to threaten global health systems and complicate the management of common infections [1, 2]. Among resistant pathogens, *Staphylococcus aureus,* particularly its methicillin-resistant strains, remains a major cause of community-acquired and hospital-acquired infections [3]. The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) have further increased the burden associated with this species. MRSA is responsible for a wide range of skin and soft-tissue infections, including wounds, abscesses, boils, and infected pus [4, 5]. These infections often progress rapidly and require prolonged treatment. Their management is becoming more difficult due to the declining effectiveness of conventional antibiotics. In this context, many people in Africa continue to use traditional medicine as a first-line approach to treat infectious diseases, using a wide range of plants known for their therapeutic properties [6, 7].

Medicinal plants represent an important source of therapeutic compounds. They contain diverse groups of secondary metabolites with documented antimicrobial, antioxidant, and anti-inflammatory activities [8, 9]. Many communities in Africa and other regions rely on plant-based remedies to manage infections when access to conventional drugs is limited [10]. Plants of the *Combretaceae* family have long been used in traditional medicine, and their therapeutic properties are described in scientific research [11] (De Morais Lima *et al*., 2012). Among them, *Combretum aculeatum* is a species that is attracting growing interest due to its traditional use in the treatment of infectious diseases [12, 13]. The plant is widely distributed across tropical regions and is used in traditional medicine to treat fever, wounds, skin infections, and gastrointestinal disorders. Phytochemical studies have shown that its biological activities are often linked to the presence of compounds such as flavonoids, tannins, phenolic acids, and terpenoids [11] (De Morais Lima *et al*., 2012). However, scientific data on its activity against clinically important resistant pathogens are limited. Few studies have investigated the phytochemical composition of its leaves or the link between its bioactive constituents and its therapeutic potential. There is also limited information on its antioxidant capacity, even though antioxidant properties are often associated with the ability of plant extracts to protect tissues and enhance wound healing [14, 15].

Antioxidant activity is of particular interest when evaluating plant extracts used to treat skin and soft tissue infections [16]. Oxidative stress can contribute to tissue damage and delay the healing of infected wounds [17]. Extracts with high antioxidant potential can help reduce oxidative stress while enhancing antimicrobial action. Flavonoids and polyphenols are among the most relevant compound classes in this context because they can scavenge free radicals, modulate inflammatory pathways, and disrupt microbial structures [18]. Quantifying these compounds can therefore provide useful information about the plant's pharmacological value. Given the growing need for new antimicrobial agents and the ethnomedicinal importance of *C. aculeatum*, an evaluation of its phytochemical compounds and antimicrobial potential is necessary.

The present study investigates the bioactivity of ethanolic and ethyl acetate extracts of *C. aculeatum* leaves. First, a phytochemical screening was carried out to identify the major groups of secondary metabolites present in the plant. The total flavonoid and polyphenol contents were quantified in order to assess the richness of the extracts in key antioxidant compounds. Antioxidant activity was then evaluated using standard *in vitro* tests to determine the extracts' ability to neutralize free radicals. Finally, the antimicrobial activity of extracts was tested against clinical isolates of MRSA obtained from wounds, abscesses, pus, and boils.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Authentication

Fresh leaves of *C. aculeatum* were collected in October 2025 from the municipality of Natitingou in

northern Benin. The botanical identification was performed at the National Herbarium of the University of Abomey-Calavi, Benin, where the species was authenticated. The certification was recorded under voucher No. YH002343/HNB. The authenticated specimen was deposited at the herbarium for future reference.

2.2. Extraction

The harvested leaves were air-dried at room temperature in the laboratory for two weeks, then ground into a fine powder. For each extraction, 50 g of the plant powder were macerated in 500 mL of solvent in a 1000 mL conical flask. One portion was extracted with 96% ethanol and the other with ethyl acetate. The mixtures were shaken for 48 hours and then filtered successively with cotton wool and Whatman No.1 paper to remove insoluble residues [19]. Each filtrate was concentrated under reduced pressure using a rotary evaporator set at 35˚C - 40˚C, 80 - 120 rpm, and 150 - 300 mbar, depending on the solvent. The concentrates obtained were dried to constant weight at 40˚C in an oven, then stored at 4˚C in sterile vials for subsequent analysis.

2.3. Phytochemical Analysis

2.3.1. Qualitative Analysis

Qualitative phytochemical screening was carried out to detect the presence of alkaloids, polyphenols, flavonoids, tannins, anthocyanins, quinones, saponins, triterpenoids, steroids, coumarins, mucilages, reducing compounds, anthracene derivatives, and cardiac glycosides in the powdered leaves of the plant following standard procedures described by Chinnadurai *et al.* [20] with slight modifications. Alkaloids were assessed using acidic extraction followed by Mayer and Bouchardat reagents. Polyphenolic compounds were evaluated from an aqueous extract, with tannins detected through ferric chloride and Stiasny tests, and flavonoids identified by the Shinoda reaction. Anthocyanins and leucoanthocyanins were screened through colorimetric changes after acidification or heating. Quinone derivatives were examined using the Born-Träger reaction. Saponins were identified by the formation of persistent foam after agitation of a diluted decoction. Triterpenoids and steroids were detected following sequential liquid-liquid extraction and characteristic color reactions. Cyanogenic derivatives were screened by maceration and exposure of released vapors to picric acid paper. Mucilages were detected by precipitation with absolute ethanol, and coumarins by fluorescence under UV light after alkaline treatment. Reducing compounds were assessed using Fehling's reagent. Anthracene derivatives were evaluated through chloroform extraction and acid hydrolysis followed by colorimetric reactions in alkaline medium. Cardiac glycosides were detected in ethanolic extracts using the Baljet, Kedde, and Raymond-Marthoud reagents.

The presence of each compound class was recorded based on characteristic precipitates, color formation, fluorescence, or foam stability [21].

2.3.2. Quantitative Analysis

❖ **Determination of Total Polyphenols**

Total polyphenol content was assessed using the Folin-Ciocalteu method. Briefly, 200 µL of each extract or gallic acid standard (prepared in methanol at appropriate dilutions) was mixed with 1 mL of Folin–Ciocalteu reagent diluted tenfold. After a 4-minute reaction time, 800 µL of sodium carbonate solution (75 mg/mL) was added. The mixture was then incubated for 2 hours at room temperature, and the absorbance was read at 760 nm. Polyphenol levels were calculated from the calibration curve ($y = 0.0013x + 0.0714$; Pearson's $R^2 = 0.9943$) obtained with gallic acid and expressed as micrograms of gallic acid equivalents per 100 mg of extract (µg GAE/mg) [22].

❖ **Determination of Total Flavonoids**

Flavonoid content was evaluated using the aluminium chloride colorimetric method. In this assay, 1 mL of a 2% $AlCl_3$ solution prepared in methanol was mixed with 1 mL of the extract or rutin standard. After allowing the reaction to proceed for 10 minutes at room temperature, absorbance was recorded at 415 nm. Flavonoid concentrations were calculated from the calibration curven ($y = 0.0025x + 0.0465$; $R^2 = 0.9928$)

generated with quercetin and expressed as micrograms of quercetin equivalents per mg of extract (μg QE/mg) [22].

❖ **Determination of total condensed tannins content**

To determine the condensed tannin content, the method described by Wendkouni *et al.* has been used [23]. Briefly, 1 mL of sample, a 10-times diluted extract (1 mg/mL), was mixed with 2 mL of 1% vanillin (comprising 1 g of vanillin and 100 mL of 70% sulfuric acid) and incubated for 15 min in a water bath at 20˚C. The absorbance of the mixture was measured at 500 nm using a BioMATE 3S UV–visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The condensed tannin content of the samples was determined in triplicate, and the results were expressed as micrograms of catechin equivalents (μg CE) per mg of extract, calculated using a catechin calibration curve ($y = 0.0003x + 0.0062$; $R^2 = 0.99$) plotted with catechin concentrations ranging from 100 to 1000 μg/mL.

The total condensed tannin content (TCT) was calculated using Formula (1):

$$\text{TCT} = X \times V_e / m_e \tag{1}$$

where X = extract solution concentration in total condensed tannins content (μg CE/mL); V_e = volume of extract used; m_e = mass contained in the volume of extract used (mg).

❖ **Determination of total hydrolysable tannins content**

Total hydrolysable tannins were measured using the method of Mole and Waterman [24]. A total of 1 mL of a 10-fold diluted extract (1 mg/mL) was mixed with 3.5 mL of reagent (10^{-2} M ferric chloride $FeCl_3$ in 10^{-3} M hydrochloric acid HCl). The absorbance was measured with a BioMATE 3S UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), at 660 nm after 15 min of incubation. The hydrolysable tannin content of the samples was determined in triplicate, and the results are expressed as μg gallic acid equivalent (GAE) per g of dried extract. The total hydrolysable tannin content (THT) was calculated according to Formula (2):

$$\text{THT} = A \times MW \times V_e / \varepsilon_{mole} \times m_e \tag{2}$$

where A = absorbance; MW = weight of gallic acid (170.12 g/mol); V_e = volume of extract; $\varepsilon_{\text{mole}}$ = 2169 (gallic acid equivalence constant); m_e = mass contained in the volume of extract used (mg).

2.4. Evaluation of Antioxidant Activity

The antioxidant potential of the ethanolic and ethyl acetate extracts was assessed using two complementary assays. The first was the DPPH (2,2-diphenyl picryl-hydrazyl) free radical scavenging test, and the second was the phosphomolybdenum reduction assay. For both methods, the activity was expressed in μmol AAE/mg of extract.

2.4.1. DPPH Radical Scavenging Test

C. aculeatum extract's scavenging property was assessed using the methodology described by fall *et al.* with some modification [14]. The assay is based on the ability of antioxidants to reduce the purple DPPH radical to a yellow-colored form, with the decrease in color intensity reflecting the scavenging capacity of the tested sample [25]. A stock solution of each extract was prepared at 400 μg/mL, and ten successive two-fold dilutions were made in methanol to obtain 1 mL of each dilution. To every dilution, 1 mL of DPPH solution (40 μg/mL in methanol) was added. A negative control containing only methanol and DPPH solution was prepared in parallel. After incubation for 20 minutes in the dark at room temperature, absorbance was measured at 517 nm against a methanol blank prepared for each concentration. Ascorbic acid was used as the reference antioxidant and processed under the same conditions [14]. All measurements were performed in triplicate. The percentage of DPPH inhibition was calculated using the following formula:

$$\text{IP} = \frac{\text{Abs}(\text{blank}) - \text{Abs}(\text{extract})}{\text{Abs}(\text{blank})} \times 100 \tag{3}$$

IP = Inhibition percentage of DPPH; Abs=Absorbance

A standard curve ($y = 8.5015\ln(x) + 97.896$; $R^2 = 0.7147$) was created using a range of ascorbic acid concentrations, with concentrations on the x-axis and inhibition rate on the y-axis. The scavenging activity (Sa) was expressed as micromoles of Ascorbic Acid Equivalent (μMol AAE) per mg of dried extract using Formula (4):

$$Sa = X \times V_e / m_e \quad (4)$$

where X = scavenging activity (mM AAE); V_e = volume of extract used; m_e = mass contained in the volume of extract used (mg).

2.4.2. Phosphomolybdenum Reduction Assay

The phosphorus molybdenum assay was performed using the protocol outlined by Kedir *et al.* [26]. We prepared an aliquot of 0.1 mL of a 10-fold diluted extract (1 mg/mL) in triplicate assay tubes. Each aliquot was treated with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95˚C in a water bath for 90 min. After cooling to room temperature, the absorbance was measured at 765 nm using a BioMATE 3S UV–visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Ascorbic acid served as the positive control to generate a standard curve ($y = 10.25\ln(x) + 84.832$; $R^2 = 0.9277$). The reducing activity was calculated from the standard curve and expressed as micromoles of Ascorbic Acid Equivalent (mMol AAE) per milligram of dried extract using Equation (5):

$$Ra = X \times V_e / m_e \quad (5)$$

where X = reducing activity (mM AAE); V_e = volume of extract used; m_e = mass contained in the volume of extract used (mg).

2.5. Antimicrobial Activity

2.5.1. Microbial Strains

A total of fifty-four clinical isolates of MRSA, isolate from pus, wounds, boils, and abscesses, were used in this study. All strains belong to the collection of the Biology and Molecular Typing Laboratory at the University of Abomey-Calavi, Benin. Each isolate was first subcultured on Mueller-Hinton (MH) agar and incubated at 37˚C for 16-20 hours to obtain fresh and pure colonies. From these cultures, bacterial suspensions were prepared by emulsifying isolated colonies in 1 mL of sterile distilled water. The turbidity of each inoculum was adjusted to match the 0.5 McFarland standard ($1 - 2 \times 10^8$ CFU/mL), which served as the reference for all antimicrobial tests. The standardized inoculum was then uniformly spread over the surface of MH agar plates using sterile cotton swabs [27].

2.5.2. Agar Disk Diffusion Method

Antimicrobial activity of the ethanolic and ethyl acetate extracts of *C. aculeatum* was assessed using the agar disk diffusion method, following a protocol adapted from Chabi-Sika *et al.* [28]. For each test, the surface of MH agar plates previously inoculated with standardized microbial suspensions was allowed to dry briefly. Four to five sterile paper disks (5 mm diameter) were then placed aseptically on each plate.

The plant extracts were prepared 24 hours before testing by dissolving 20 mg of crude extract in 1 mL of sterile distilled water. Each disk was impregnated aseptically with 30 µL of the extract solution. The loaded disks were allowed to diffuse into the agar for approximately 15-30 minutes at room temperature before incubation. All plates were incubated at 37 ˚C, and inhibition zones were measured in mm after 24 hours and 48 hours [27, 28].

2.6. Statistical Analysis

The data were recorded in an Excel spreadsheet. Phenolic content and antioxidant activity were visualized using bar charts and statistically compared with Welch's t-test in GraphPad Prism 10. Antibacterial

activity data were also represented with bar charts, generated in R Studio. All statistical analyses were conducted at a 95% confidence level. Principal component analysis was performed to explore the relationships among bacterial susceptibility, extracts, and their sources

3. RESULTS

3.1. Phytochemical Screening

✓ **Main classes of specialized metabolites**

Table 1 summarizes the qualitative phytochemical screening of *C. aculeatum* leaves. The results indicate the presence of several major classes of specialized metabolites. Polyphenolic compounds were detected such as gallic tannins and catechetical tannins. Flavonoids were present, along with leucoanthocyanins, while anthocyanins and coumarins were not detected. Quinonic derivatives, saponosides, mucilage, reducing compounds, and alkaloids were also identified. In contrast, triterpenoids and cyanogenic derivatives were absent. Indeed, the phytochemical screening reveals that *C. aculeatum* leaves contain a broad range of secondary metabolites, with a predominance of polyphenolic compounds and the presence of several other bioactive classes.

Table 1. Qualitative phytochemical profile of *C. aculeatum* leaves.

Classes		specialized metabolites	*C. aculeatum*
Polyphenols	Tannins	Gallic tannins	+
		Catechetical tannins	+
	Flavonoids	Flavonoids	+
		Anthocyanins	–
		Leucoanthocyanins	+
	Coumarines		–
Quinonic derivatives			+
Saponosides			+
Terpene derivatives		Triterpenoids	–
Cyanogenic derivatives			–
Mucilage			+
Reducing compounds			+
Alkaloids			+

Captions: + = presence; − = absence.

✓ **Phenolic content of the extracts**

Figure 1 presents a comparative quantitative assessment of the major phenolic constituents in the ethanolic and ethyl acetate extracts of *C. aculeatum* leaves. Total phenolic content (a) was comparable between the two extracts, with values of 148.27 µg GAE/mg for the ethanolic extract and 142.38 µg GAE/mg for the ethyl acetate extract, showing no statistically significant difference ($p > 0.5$). In contrast, total flavonoid content (b) was significantly higher in the ethanolic extract (56.95 µg QE/mg) than in the ethyl acetate extract (35.14 µg QE/mg) ($p < 0.5$). The levels of condensed tannins (c) were similar in both extracts, with

33.45 µg CE/mg for the ethanolic extract and 31.11 µg CE/mg for the ethyl acetate extract, and no significant difference was observed ($p > 0.5$). Hydrolysable tannins (d) were present at higher levels in the ethanolic extract (2.24 µg GAE/mg of dried extract), and this difference was statistically significant compared to the ethyl acetate extract ($p < 0.5$). The figure indicates that both solvents extract comparable amounts of total phenolics and condensed tannins, whereas ethanol is more effective for the extraction of flavonoids and hydrolysable tannins.

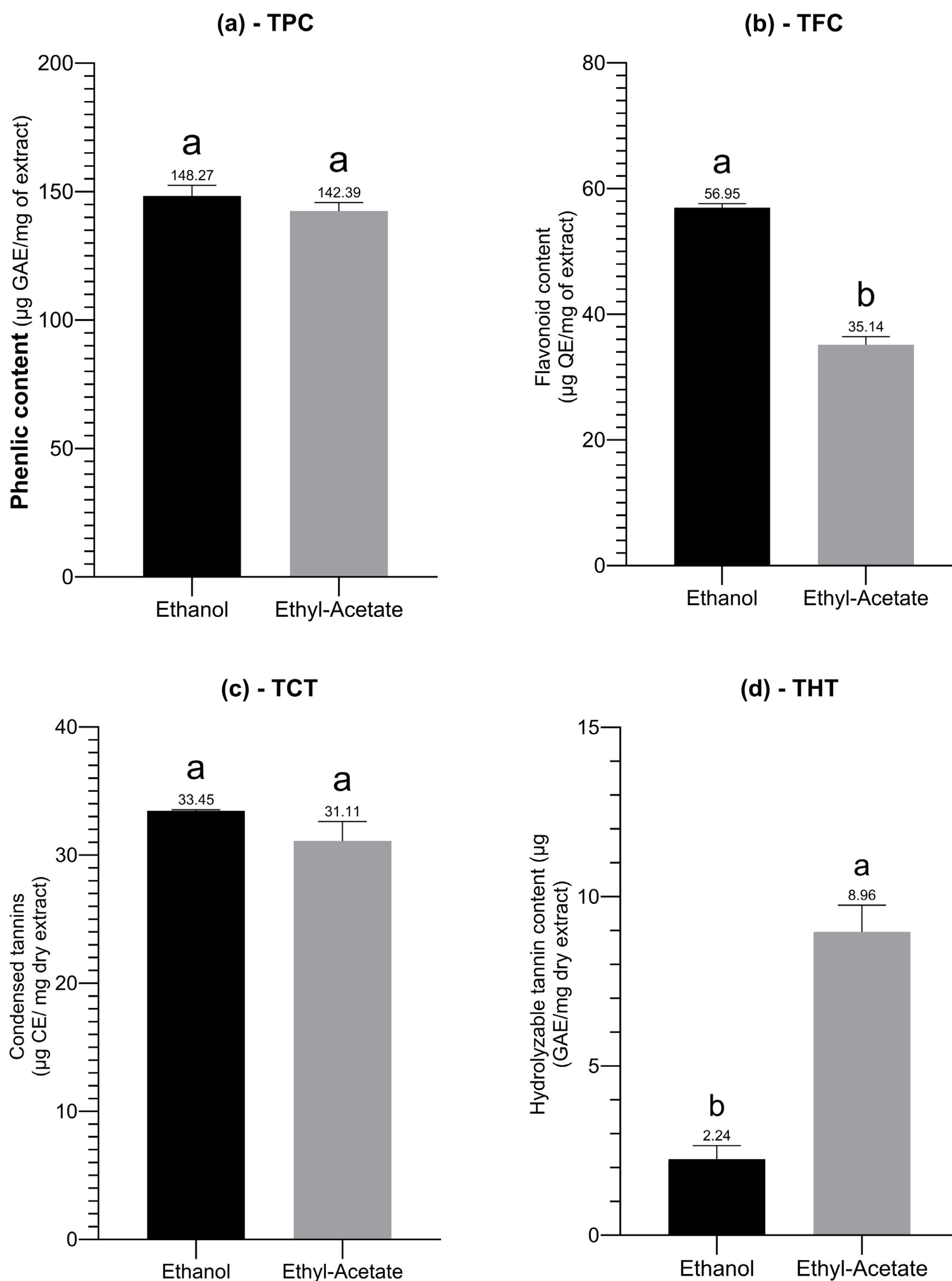


Figure 1. Phenolic composition of *C. aculeatum* ethanolic and ethyl acetate extracts: (a) Total phenolic content (TPC); (b) Total flavonoid content (TFC); (c) Total condensed tannins content (TCT), and (d) total hydrolysable tannins content (THT). Ţ: Standard Deviation.

3.2. Antioxidant Activity of the Extracts

Figure 2 illustrates the antioxidant activity of the ethanolic and ethyl acetate extracts of *C. aculeatum* leaves as evaluated by the phosphomolybdenum reduction assay and the DPPH radical scavenging test. In the phosphomolybdenum reduction assay (a), both extracts exhibited comparable total antioxidant capacity, with values of 122.96 mMol AAE/mg for the ethanolic extract and 123.04 mMol AAE/mg for the ethyl acetate extract. Statistical analysis indicated no significant difference between the two extracts. In the DPPH radical scavenging assay (b), the ethanolic extract showed a higher mean activity (7.45 µMol AAE/mg) than the ethyl acetate extract (3.45 µMol AAE/mg); however, this difference was not statistically significant. Both ethanolic and ethyl acetate extracts shows comparable antioxidant capacities in terms of phosphomolybdenum reduction assay and free radical scavenging activity under the tested conditions.

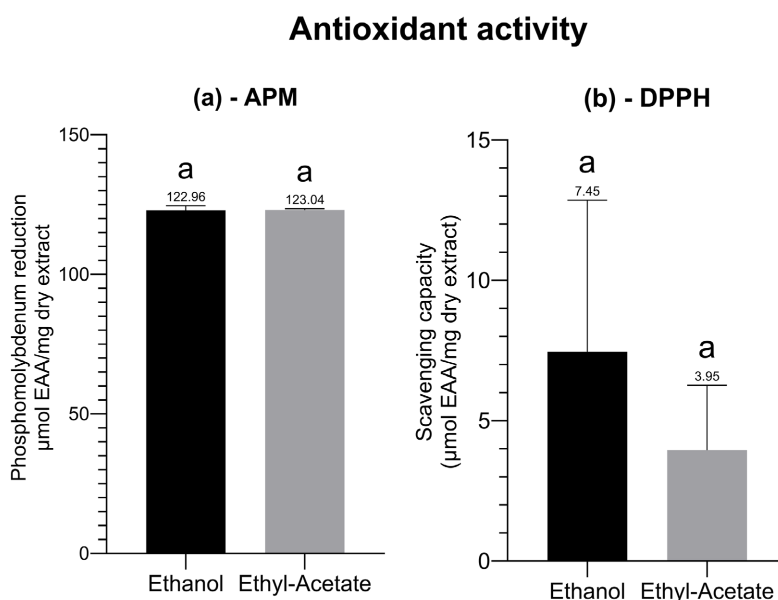


Figure 2. Antioxidant activity of the ethanolic and ethyl acetate extracts of *C. aculeatum* leaves: (a) APM phosphomolybdenum reduction assay and (b) DPPH radical scavenging test. Ţ: Standard Deviation.

3.3. Antibacterial Activity

Figure 3 presents the mean inhibition diameters of the ethanolic and ethyl acetate extracts of *C. aculeatum* against MRSA strains isolated from abscesses, boils, wounds, and pus. Both extracts exhibited inhibitory activity against all tested strains. After 24 hours of incubation, inhibition diameters ranged from 23.32 to 27.00 mm for the ethanolic extract and from 15.67 to 24.08 mm for the ethyl acetate extract. At 48 hours, inhibition zones for the ethanolic extract varied between 23.58 and 26.00 mm, while those of the ethyl acetate extract ranged from 15.74 to 24.24 mm. The ethanolic extract showed greater activity against strains isolated from abscesses, wounds, and pus, whereas both extracts displayed comparable activity against strains isolated from boils. Statistical analysis indicated no significant difference between inhibition diameters recorded at 24 and 48 hours for either extract ($p > 0.5$).

3.4. Relationship between Susceptibility of MRSA Strains, the Extracts and the Source of the Strains

Figure 4 shows the relationship between the susceptibility of MRSA clinical strains, the plant extracts, and the source of isolation using principal component analysis (PCA). The PCA biplot (a) shows the distribution of the variables and strain sources along the principal components, highlighting the associations

between extract activity and strain origin. The projection indicates that the susceptibility patterns of the strains vary according to both the type of extract and the clinical source, suggesting heterogeneity in strain responses. The individual plot (b) presents the dispersion of MRSA strains according to their susceptibility profiles. Strains isolated from wounds, pus, abscesses, and boils are distributed across different regions of the factorial space, reflecting variability in their responses to the tested extracts. The variables plot (c) displays the contribution of the extracts and strain sources to the principal components. The positioning of variables indicates their relative influence on strain susceptibility and highlights the relationships between extract type and the clinical origin of the isolates. In general, it appears that the susceptibility of MRSA strains is structured according to both the plant extracts and the source of isolation, as revealed by the multivariate analysis.

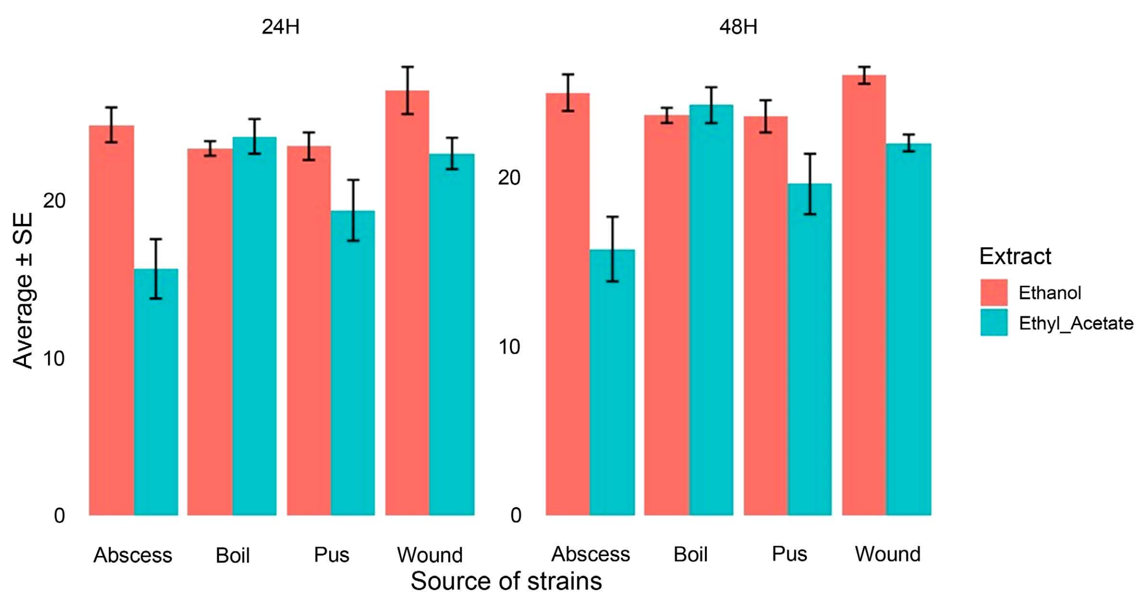
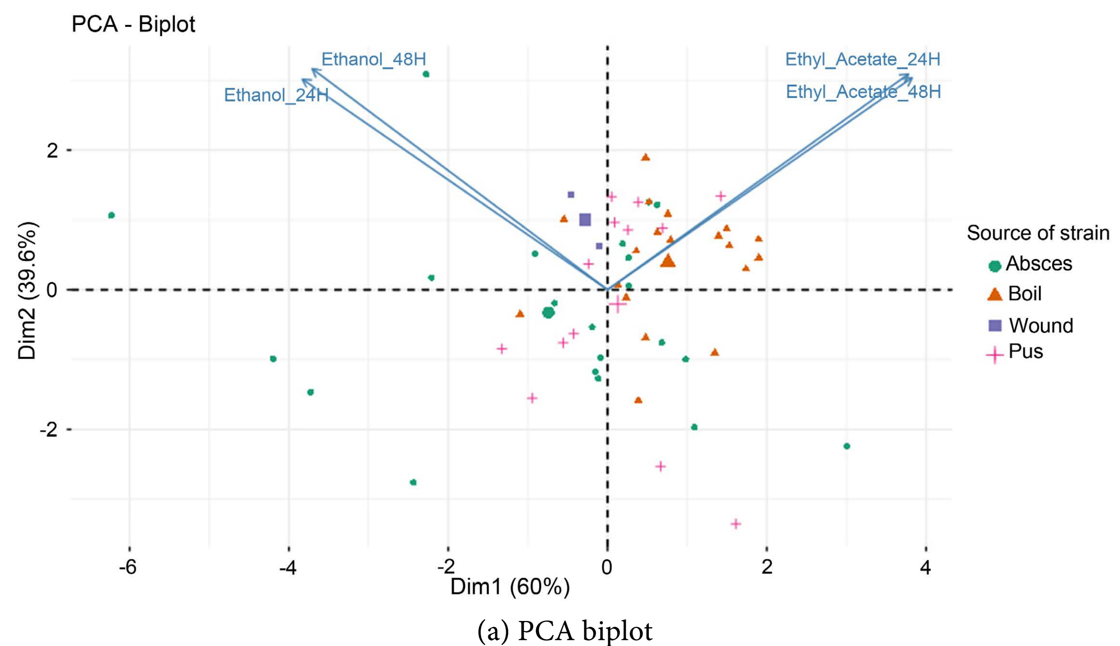
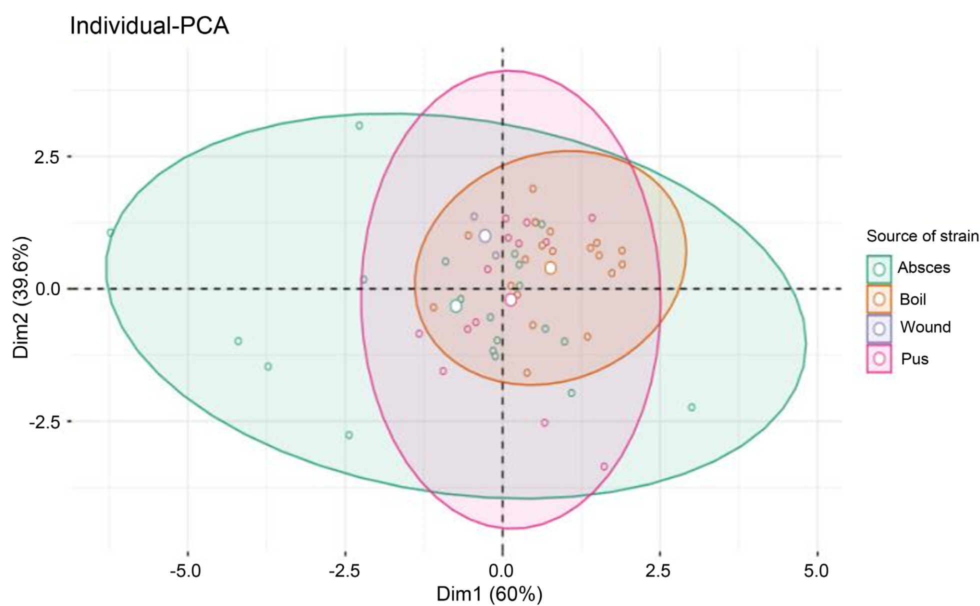


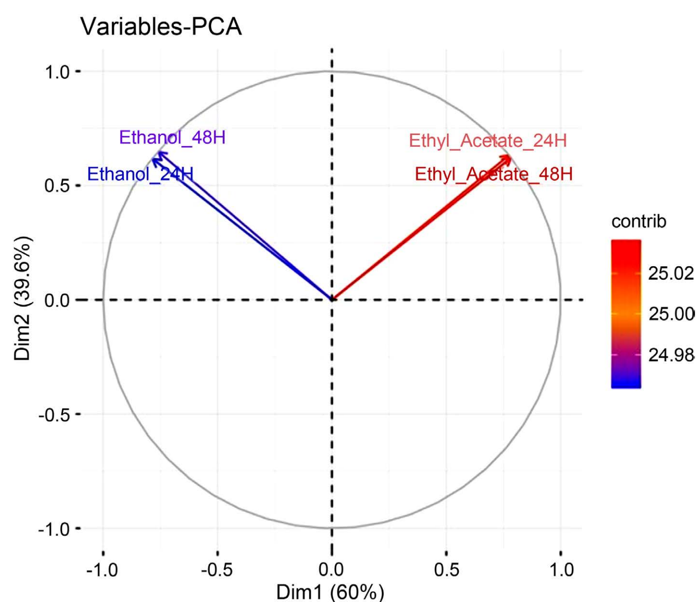
Figure 3. The mean inhibition diameters of *C. aculetum* ethanol and ethyl acetate extracts on MRSA after 24 h (a) and 48 h (b). Ţ: Standard Deviation.



(a) PCA biplot



(b) Individual



(c) Variables

Figure 4. Principal component analysis (PCA) showing the relationship between MRSA strain susceptibility, extract type, and source of isolation.

4. DISCUSSION

The qualitative phytochemical screening revealed that *C. aculeatum* leaves are rich in diverse classes of specialized metabolites, notably polyphenols, flavonoids, tannins, quinonic derivatives, saponosides, alkaloids, mucilage, and reducing compounds. The predominance of polyphenolic constituents observed in this study is consistent with previous reports on species of the *Combretum* genus, which are recognized as important reservoirs of bioactive phenolic compounds [11, 13]. The absence of cyanogenic derivatives and triterpenoids suggests a favorable safety profile and a phytochemical composition oriented toward antioxidant and antimicrobial activities. Quantitative analyses confirmed the richness of both ethanolic and ethyl

acetate extracts in phenolic compounds. Total phenolic content and condensed tannins were comparable between the two extracts, indicating that both solvents efficiently extracted these compound classes. In contrast, ethanol proved significantly more effective in extracting flavonoids and hydrolysable tannins. This observation aligns with the higher polarity of ethanol, which favors the solubilization of hydroxylated phenolics and glycosylated flavonoids [22, 27]. These results suggest that solvent polarity plays a key role in shaping the phenolic profile and, consequently, the biological activities of the extracts.

The extracts showed antioxidant capacity as demonstrated by the phosphomolybdenum reduction assay and the DPPH radical scavenging test. The absence of a significant difference in total antioxidant capacity between extracts in the phosphomolybdenum assay indicates that the global reducing potential is comparable, despite differences in specific phenolic subclasses. This assay reflects cumulative electron-donating ability rather than the contribution of individual compounds [26]. The ethanolic extract showed higher mean DPPH scavenging activity, which is consistent with its significantly higher flavonoid and hydrolysable tannin contents. Flavonoids are well known for their capacity to donate hydrogen atoms and neutralize free radicals, thereby contributing to radical scavenging activity [25]. Similar associations between flavonoid richness and antioxidant capacity have been reported for *C. aculeatum* and related species [14, 15].

The antimicrobial evaluation demonstrated that ethanolic and ethyl acetate extracts inhibited the growth of all tested MRSA clinical isolates, regardless of their source. This broad activity spectrum is particularly important given the clinical relevance of MRSA as a multidrug-resistant pathogen involved in skin and soft tissue infections [3, 4]. The comparable inhibition diameters observed at 24 h and 48 h indicate that the antibacterial effect is stable over time and not transient. The higher activity of the ethanolic extract against strains isolated from wounds, pus, and abscesses may be attributed to its higher flavonoid and hydrolysable tannin contents. Polyphenols and tannins can disrupt bacterial cell walls, precipitate proteins, inhibit enzymes, and interfere with quorum sensing mechanisms [16, 18]. Alkaloids and quinones detected in the leaves may further contribute through DNA intercalation and redox cycling. The lack of data on the Minimum Inhibitory Concentration (MIC) is a limitation of this study. Indeed, disc diffusion provides only preliminary evidence of sensitivity.

The principal component analysis provided additional insight into the relationships between extract type, strain origin, and susceptibility profiles. The dispersion of strains according to their clinical source highlights the heterogeneity of MRSA responses, which is expected given the genetic and phenotypic diversity of clinical isolates [5]. The PCA indicates that both extract type and strain origin contribute to the observed variability, supporting the importance of testing multiple clinical isolates rather than reference strains alone. This multivariate approach strengthens the translational value of the findings and supports the antibacterial evaluation.

5. CONCLUSION

This study demonstrates that *C. aculeatum* leaves are a rich source of bioactive secondary metabolites, particularly polyphenols and flavonoids. Ethanolic and ethyl acetate extracts exhibited comparable total phenolic content and antioxidant capacity, while ethanol was more effective in extracting flavonoids and hydrolysable tannins. Both extracts showed antibacterial activity against clinical MRSA isolates from diverse infection sources, highlighting their potential in the management of skin and soft tissue infections. The observed bioactivities are supported by the phytochemical composition and reinforced by multivariate analysis of strain susceptibility. These findings provide scientific support for the traditional use of *C. aculeatum* and justify further studies focused on compound isolation, mechanism of action, and in vivo validation.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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