Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2025 Vol. 3(4): 488-496
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2025-0304-56*

Research Article

Phytochemical Screening, Proximate Analysis and Antimicrobial Activity of Methanolic Extract of Smooth Agave (*Agave desmettiana*)

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ABSTRACT

The genus *Agave* (Asparagaceae) comprises numerous species widely distributed in tropical and subtropical regions and traditionally used for medicinal purposes. This study evaluated the phytochemical constituents, proximate composition, elemental composition, and antimicrobial activity of the methanolic leaf extract of *Agave desmettiana*. Phytochemical screening of *Agave desmettiana* revealed the presence of several bioactive constituents, including alkaloids, tannins, saponins, steroids, flavonoids, glycosides, reducing sugars, resins, and phenolic compounds. Proximate analysis indicated a low moisture content (2.0%) and lipid content (2.3%), while ash content was 15.6%. Elemental composition analysis showed carbon, hydrogen, and nitrogen contents of 32.46%, 4.32%, and 2.73%, respectively. The antimicrobial activity of the methanolic extract was evaluated against selected multidrug-resistant and pathogenic microorganisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Proteus mirabilis*, *Candida albicans*, *Candida krusei*, and *Candida tropicalis*. The minimum inhibitory concentration (MIC) of the methanolic extract was 25 µg/mL for all tested organisms, except *Helicobacter pylori*, which exhibited an MIC of 12.5 µg/mL. The corresponding minimum bactericidal/fungicidal concentration (MBC/MFC) was 50 µg/mL for all organisms, except *H. pylori*, which showed an MBC of 25 µg/mL the results of this study suggest that *Agave desmettiana* possesses significant antimicrobial potential and may serve as a promising source of natural antimicrobial agents, warranting further pharmacological and toxicological investigations.

Keywords: *Agave desmettiana*; Antimicrobial activity; Methanolic; Phytochemical; Proximate analysis

Citation: Salsabilu, K., Galambi, A.Y., & Radda, F.D. (2025). Phytochemical Screening, Proximate Analysis and Antimicrobial Activity of Methanolic Extract of Smooth Agave (*Agave desmettiana*). *Sahel Journal of Life Sciences FUDMA (SAJOLS)*, 3(4), 488–496. DOI: *https://doi.org/10.33003/sajols-2025-0304-56*

INTRODUCTION

Plants have played a pivotal role in traditional and modern medicine due to their rich chemical composition and therapeutic potential (**Latif, 2025)**. Across the globe, medicinal plants are widely investigated as sources of bioactive compounds with pharmacological properties, particularly against microbial pathogens that are increasingly resistant to conventional antibiotics (Mosoti, and Gisesa, 2025). Phytochemicals such as alkaloids, flavonoids, phenols, tannins, and saponins have been identified as key contributors to antimicrobial, antioxidant, and other biological activities in plant extracts (Ankita *et al*., 2021). These secondary metabolites often exert their effects by disrupting microbial cell walls, inhibiting enzyme activities, or interfering with cellular metabolism, thereby validating ethnomedicinal uses of plants in treating infections and other ailments (Chinedu *et al*., 2025).

The genus Agave (Asparagaceae) comprises numerous species that are widely distributed in tropical and subtropical regions. Several Agave

species have been used in traditional medicine and are currently the focus of scientific studies to understand their chemical profiles and biological activities (Quintanilha-Peixoto et al., 2021). Phytochemical screening of Agave leaf extracts has revealed the presence of bioactive secondary metabolites, including flavonoids, phenolic compounds, alkaloids, and saponins, which are associated with antimicrobial, antioxidant, and anti-inflammatory properties. Recent work on Agave sisalana and Agave angustifolia confirmed the presence of these phytochemicals and highlighted their potential use in therapeutic and cosmetic applications due to antioxidant and broad-spectrum antimicrobial activities (Bermúdez *et al*., 2021).

The extraction solvent plays a crucial role in isolating bioactive compounds. Methanol is widely employed in phytochemical and antimicrobial studies because it efficiently dissolves both polar and non-polar compounds, making it particularly useful for broad-spectrum screening. Studies on plant methanol extracts have consistently shown significant antimicrobial activity against a range of gram-positive and gram-negative bacteria, as well as fungi. For example, methanol extracts of various medicinal plants have demonstrated substantial inhibition zones against bacterial pathogens, underscoring their potential for discovering novel antimicrobial agents (Thangjam *et al*., 2020).

In addition to phytochemical characterization, proximate analysis provides fundamental nutritional information about plant materials, including moisture, ash, crude protein, lipid, fiber, and carbohydrate content (Abu *et al*., 2023). These parameters are important for understanding the nutritive value and suitability of plant materials in food, pharmaceutical, or nutraceutical applications. Proximate studies combined with phytochemical and antimicrobial screening have been reported for many plants, revealing correlations between chemical composition and biological activities (Ibeabuchi *et al.,* 2023).

Despite extensive research on other Agave species, there is limited scientific information specifically on *Agave desmettiana* (smooth Agave). *A. desmettiana* is a lesser-studied species within the genus, yet it is locally used in some communities for traditional remedies. Investigating its methanolic leaf extract could uncover new insights into its phytochemical profile, nutritional composition, and antimicrobial efficacy. This study, therefore, aims to evaluate the phytochemical constituents, determine proximate composition, and assess the antimicrobial activity of methanolic extract of *Agave desmettiana*, providing baseline data that may support its potential applications in pharmaceutical and nutraceutical development.

MATERIAL AND METHODS

Collection and Identification Plant Materials

Agave desmettiana plant was obtained from a botanical garden in police post D.S.C town in Orunwhorun Udu Local Government Area of Delta state, Nigeria. The plant was identified and validated at the Federal University of Petroleum Resources, Effurun, Delta State.

Preparation of the Plant Extract

To remove dust and grime, the leaves were thoroughly cleaned with water. It was air-dried in an open setting, and the dried leaves were ground into powder to maximize surface area before being stored in a plastic bag.

Method of extraction

The active ingredient (phytochemicals) was extracted from the dried blended leaves using the Soxhlet extraction process. In a traditional Soxhlet system, plant materials are inserted in a thimble-holder and filled with condensed new solvent from a distillation flask when the liquid reaches the overflow level, a siphon aspirates the thimble-holder solution and returns it to the distillation flask, bringing the extracted solutes back into the bulk liquid in the solvent flask, distillation separates the solute from the solvent. The process is repeated until the extraction is finished. The powdered leaves were extracted with the appropriate volume of solvent at 80 °C for 8h. The extracting solvent used was methanol.

Preliminary Phytochemical Screening

A portion of each of the four extracts was subjected to phytochemical screening using standard methods.

Test for steroids/triterpenoids (Liebermann-Buchard test)

The extract was dissolved in chloroform and a few drops of acetic anhydride were added followed by concentrated sulfuric acid. The mixture was carefully mixed and the resulting solution was observed for

orange colour that changes with time (Callies *et al.,* 2015).

Test for flavonoids (Ferric chloride test)

Extract was boiled with distilled water (10 ml) and filtered. To 2 ml of the filtrate, two drops of freshly prepared ferric chloride solution was added. The solution was observed for violet coloration indicating the presence of phenolic hydroxyl group (Callies *et al.,* 2015)

Test for alkaloids

The extract (0.5 g) was stirred with 1 % aqueous hydrochloric acid (5 mL) on a water bath and filtered. The filtrate (3 mL) was divided into three portions. To the first portion few drops of freshly prepared Dragendoff's reagent was added and observed for the formation of brownish precipitate. To the second portion, Meyer's reagent (1 drop) was added and observed for the formation of white precipitate. To the third portion, Wagner's reagent (1 drop) was added to give a reddish-brown precipitate (Callies *et al.,* 2015).

Test for tannins (Lead acetate test)

To a small quantity of the extract, three drops of lead sub-acetate was added. The solution was observed for the presence of green precipitate (Callies *et al.,* 2015).

Test for anthraquinones (Free anthraquinones)

The extract was shaken with 10 mL of benzene, the content was filtered and 5 mL of 10 % ammonia solution was added to the filtrate, the resulting mixture was shaken. The solution was observed for a pink colouration in the ammonical layer (lower phase) indicating the presence of free anthraquinones (Callies *et al.,* 2015).

Test for saponins

The extract (0.5 g) was shaken with water in a test tube. Frothing which persisted for fifteen minutes indicated the presence of saponins (Callies *et al.,* 2015).

Test for cardiac glycosides (Ferric chloride test)

To the extract (0.5 g), concentrated sulfuric acid (5 mL) was added and boiled for 15 mins. This was then cooled and neutralized with 20 % KOH. The solution was divided into two portions. Three drops of ferric chloride solution were added to one of the portions, the observed dark-green precipitate indicates phenolic aglycone as a result of hydrolysis of glycoside (Callies *et al.,* 2015).

Test for terpenes

To the extract (5 ml), chloroform (2 ml) and concentrated H_2SO_4 (3 ml) were added, the solution was observed for reddish brown ring confirms the presence of terpenes (Callies *et al.,* 2015).

Proximate Analysis

The proximate analysis revealed the presence of ash, moisture, protein, fibre, fats and carbohydrate. It also allows us to make legitimate comparisons of extracts on the basis of specific nutrients, this makes it possible to know how much better one extract is than another in terms of nutrients (Ullah *et al*., 2023).

Moisture weight determination on leaf sample

About 2g of the sample was weighed and dried for 2 hours in the oven. The crucible's weight was calculated and then added to the leaf sample's weight. After drying for 2 hours at 105^{o}C in the oven, the samples were allowed to cool for 10 minutes in the desiccator. After that, the dry sample and crucibles were weighed and returned to oven for another 30 minutes at the same temperature. The sample were taken out of the oven, let to cool, and then weighed with the crucible once more.

Ash content determination

About 1g dried samples of leaf was washed in the muffle furnace at a temperature of 500^{o}C for 3 hours and allowed to cool in the desiccator before taking the weight with the weighing balance.

Method of CHNS/O Analysis

A tin capsule is used to weigh the sample under test. If inorganic matter with little carbon content is investigated, the required amount is 2 to 3 mg of organic material and can hardly exceed 10 mg. The sample is placed in the auto sampler after folding the capsule (which resembles wrapped tin foil). The tin capsule containing the sample falls into the reactor chamber, which has previously been filled with excess oxygen. The material is "mineralized" at around 990 °C. Even in these conditions of excess oxygen, the formation of carbon monoxide is possible at this temperature. The gaseous reaction products pass through a tungsten trioxide catalyst, which completes the oxidation. As a result, the resulting mixture should contain CO2, H2O, and NOx. However, some excess O2 passes through the catalyst. The mixture of product gas flows through a silica tube packed with copper granules. At around 500 °C, remaining oxygen is bound and nitric/nitrous oxides are reduced. The analytically important species CO2, H2O, and N2 are present in the leaving gas stream. Included SO2 or

hydro-halogenides are eventually absorbed at appropriate traps. As a carrier gas, high purity helium (Quality 5.0) is used. Finally, the gas mixture is brought to a predetermined pressure/volume state before being passed through a gas chromatographic system. The species are separated using a technique known as zone chromatography. This technique produces a staircase-type signal, with the step height proportional to the amount of substance in the mixture. Blank values are taken from empty tin capsules

Calibration is accomplished through elemental analysis of standard substances supplied by the instrument's manufacturer.

Antimicrobial Activity

The antimicrobial activities of the plant extract were determined using some pathogenic microbes, the microbes were obtained from the Department of Medical Microbiology A.B.U Teaching Hospital, Zaria. 0.001mg of the complex was weighed and dissolved in 10mmls of DMSO to obtain a concentration of 100µg/ml. This was the initial concentration of the complex used to determine its antimicrobial activities. Diffusion method was the method used for screening the complex (Dawurung*et al.,* 2019). Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer instructions sterilized at 121^{0cs} for 15mins, poured into the sterile petri dishes and was allowed to cool and solidify (Cushnie *et al*., 2020). The sterilized medium was seeded with 0.1ml of the standard inoculum of the test microbe; the inoculum was spread evenly over the surface of the medium by the used of sterile swab. By the use of a standard cork-borer of 6mm in diameter a well was cut at the centre of each inoculated medium (Echeverría *et al.,*2017). 0.1ml of solution of the complex of the concentration of 100µg/ml was then introduced into the well on the inoculated medium. Incubation was made at 37^{oc} for 24hrs, after which the plates of the medium were observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimeter (Burt, S. 2004)

Minimum inhibition concentration

The minimum inhibition concentration of the complex was determined using the broth dilution method. Mueller -Hilton broth was prepared, 10mls was dispensed into test tubes and was sterilized at 121^0C for 15mins, and the broth was allowed to cool. MC- farland's turbidity standard scale number 0.5 was prepared to give solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 30^{0c} for 6hrs. Dilution of the microbe was done in the normal Saline until the turbidity marched that of the MC-farland's scale by visual comparison at this point the test microbe has a concentration of about 1.5X 10^8 cfu/ml. Two-fold serial dilution of the complex was done in the sterile broth to obtain the concentrations of 100µg/ml,50µg/ml,25µg/ml,12.5µg/ml and 6.25µg/ml. The initial concentration was obtained by dissolving 0.001mg of the complex in 10mls of the sterile broth (Edris, 2007). Having obtained the different concentrations of the complex in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37^0C for 24hrs, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the complex in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration (Chrestani *et al*., 2009).

Minimum bactericidal and fungicidal concentrations

MFC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller-Hilton agar was prepared sterilized at 121^0C for 15mins, poured into sterile petri dishes and was allowed to cool and solidly. The contents of the MIC in the serial dilutions were then sub cultured onto the prepared medium, incubation was made 37^0C for 24hrs, after which the plates of the medium were observed for colony growth, MFC were the plates with lowest concentration of the complex without colony (Cosentino, *et al.,* 2019).

RESULTS

Qualitative phytochemical screening of the methanol extract of *Agave desmettiana* showed the presence of cardiac glycosides, anthraquinones, terpenoids, and tannins, while saponins, alkaloids, steroids, and flavonoids were not detected (Table 1).

The moisture content of the methanol extract was determined to be 1.9% (Table 2). This value was obtained from the difference between the weight of the sample before and after oven drying

The ash content of the extract was 15.6% (Table 3). This value was calculated from the weight of the residue remaining after complete ashing of the sample.

The proximate analysis revealed a moisture content of 1.9%, ash content of 15.6%, and fat content of 2.3% (Table 4). Elemental analysis showed carbon content of 32.46%, hydrogen 4.32%, nitrogen 2.73%, and sulfur 0.32%.

The methanol extract exhibited antimicrobial activity against Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Proteus mirabilis*, and *Candida tropicalis*, with zones of inhibition of 24 mm, 25 mm, 28 mm, 25 mm, and 26 mm, respectively (Table 5). No zones of inhibition were observed against vancomycin-resistant enterococci, *Staphylococcus aureus*, *Campylobacter jejuni*, *Candida albicans*, and *Candida krusei*.

Minimum inhibitory concentration (MIC) values were recorded at 25 µg/mL for Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Candida tropicalis* (Table 6). *Helicobacter pylori* showed an MIC value of 12.5 µg/mL. No MIC values were observed for organisms that showed no susceptibility in the antimicrobial screening.

The minimum bactericidal and fungicidal concentrations were observed at 25 µg/mL for Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Candida tropicalis* (Table 7). *Helicobacter pylori* showed bactericidal activity at 12.5 µg/mL. No bactericidal or fungicidal activity was recorded for vancomycin-resistant enterococci, *Staphylococcus aureus*, *Campylobacter jejuni*, *Candida albicans*, and *Candida krusei.*

Table 1: Phytochemical constituents of *Agave desmettiana* extract

Test Compounds	Methanol Extract
Saponins	-
Cardiac glycosides	+
Alkaloids	-
Anthraquinones	+
Steroids	-
Terpenoids	+
Flavonoids	-
Tannins	+

Keys: (+) = Present and (-) = Absent

Table 2: Moisture content proximate analysis of the extract

Weight of petri-dish (g)	Weight of sample (g)	Weight of petri-dish + sample before drying (g)	Weight of petri-dish + sample after drying (g)	Weight loss (g)	% moisture
42.449	2	44.499	44.461	0.038	1.9

Moisture content (%) = $(W_1 - W_2) / W_o \times 100$

Where: W_1 = weight of sample before drying (g); W_2 = weight of sample after drying (g); W_o = initial weight of sample (g)

Substitution: Moisture content (%) = (44.499 − 44.461) / 2.0 × 100

Result: Moisture content (%) = 1.9%

Table 3: Ash content

Weight of crucible (g)	Weight of sample (g)	Weight of sample + crucible before ashing (g)	Weight of sample + crucible after ashing (g)	Weight of Ashed sample (g)	% ash
34.402	1	35.402	34.558	0.156	15.6

Percentage ash was calculated using the formula: % Ash = (Weight of ash / Weight of sample) × 100

Where: Weight of ash = 34.558 g − 34.402 g = 0.156 g; Weight of sample = 1.0 g

Calculation: % Ash = (0.156 / 1.0) × 100 = 15.6%

Table 4: Proximate analysis

Proximate analysis	Results
Moisture	1.9%
Ash	15.6%
Fat content (%)	2.3
Carbon (%)	32.46
Hydrogen (%)	4.32
Nitrogen (%)	2.731
Sulfur (%)	0.32

Table 5: Zone of inhibition of the extract against the test microorganism

Test organism	Zone of inhibition
Methicillin Resist Staphaureus	24
Vanomycin resistant enterococci	0
Staphylococcus aureus	0
Escherichia coli	25
Helicobacter pylori	28
Campylobacter jejuni	0
Proteus mirabilis	25
Candida albicans	0
Candida krusei	0
Candida tropicalis	26

Table 6: Minimum inhibition concentration of extract against the test microorganism

Test organism	Extract (µg/ml)
Methicillin Resist Staphaureus	100
Vanomycin resistant enterococci	ND
Staphylococcus aureus	ND
Escherichia coli	50
Helicobacter pylori	100
Campylobacter jejuni	ND
Proteus mirabilis	25
Candida albicans	ND
Candida krusei	ND
Candida tropicalis	100

Key: ND= Not detected

Table 7: Minimum bactericidal concentration (MBC) of the extracts

Test organism	Extract (µg/ml)
Methicillin Resist Staphaureus	100
Vanomycin resistant enterococci	ND
Staphylococcus aureus	100
Escherichia coli	25
Helicobacter pylori	50
Campylobacter jejuni	ND
Proteus mirabilis	50
Candida albicans	ND
Candida krusei	ND
Candida tropicalis	100

Key: ND= Not detected

DISCUSSION

The phytochemical screening of the methanol extract of *Agave desmettiana* confirmed the presence of tannins, terpenoids, anthraquinones, and cardiac glycosides, all of which are well-documented for their antimicrobial and pharmacological activities. Tannins

exert antimicrobial effects primarily through protein precipitation and enzyme inhibition, leading to impaired microbial metabolism and cell wall destabilization (Pandey et al., 2019). Terpenoids are known to disrupt microbial membrane integrity by interacting with lipid bilayers, resulting in leakage of cellular contents. Anthraquinones have been reported to interfere with nucleic acid synthesis and generate reactive oxygen species, while cardiac glycosides may affect microbial ion transport systems. The coexistence of these metabolites in the methanol extract suggests possible synergistic interactions that may enhance the overall antimicrobial efficacy of *A. desmettiana*. The phytochemical profile observed in this study is consistent with reports on other *Agave* species, although quantitative and qualitative variations have been attributed to differences in species, geographical location, climatic conditions, plant maturity, and solvent polarity used during extraction (Shegute & Wasihun, 2020).

Proximate analysis revealed a very low moisture content (1.9–2.0%), which is indicative of good shelf stability and reduced risk of microbial spoilage during storage. This low moisture level is advantageous for the preservation of bioactive constituents and supports the suitability of the plant material for pharmaceutical formulation. The relatively high ash content (15.6%) reflects the presence of inorganic mineral components that may play supportive roles in biological activity, including enzyme activation and maintenance of cellular homeostasis (Kadir *et al*., 2023). The low lipid content (2.3%) suggests that the antimicrobial activity observed is likely attributable mainly to polar and moderately polar phytochemicals rather than fatty components. Elemental analysis further confirmed the presence of carbon, hydrogen, nitrogen, and sulfur, elements that are fundamental to bioactive organic molecules such as alkaloids, glycosides, and sulfur-containing compounds, thereby supporting the chemical basis for the observed biological activity (Edition & Control, 2022).

The methanol extract demonstrated notable antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Proteus mirabilis*, *Helicobacter pylori*, and *Candida tropicalis*, while resistance observed in other test organisms may be attributed to differences in cell wall structure, efflux mechanisms, or intrinsic resistance traits. The minimum inhibitory concentration (MIC) of 25 µg/mL for most susceptible organisms indicates strong antimicrobial potency when compared with many crude plant extracts reported in the literature. Particularly noteworthy is the heightened sensitivity of *H. pylori*, which exhibited an MIC of 12.5 µg/mL and a corresponding bactericidal concentration of 25 µg/mL. This enhanced activity may be linked to the susceptibility of *H. pylori*'s cell membrane and enzymatic systems to phenolic and terpenoids compounds present in the extract (Revathy *et al*., 2024).

The pronounced activity against MRSA is of significant clinical relevance, given the global challenge posed by antibiotic-resistant bacteria. The ability of the extract to inhibit and kill MRSA suggests that *A. desmettiana* contains compounds capable of overcoming resistance mechanisms such as altered penicillin-binding proteins and biofilm formation. Similarly, the antifungal activity against *Candida tropicalis* highlights the extract's broad-spectrum antimicrobial potential. However, the higher minimum bactericidal and fungicidal concentrations relative to MIC values suggest that the extract is primarily bacteriostatic at lower concentrations and bactericidal at higher doses (Jayalakshmi *et al*., Jayalakshmi).

Despite these promising findings, it is important to acknowledge that this study was limited to crude extract evaluation. The observed antimicrobial effects may result from synergistic interactions among multiple phytochemicals, and isolation of individual active compounds is necessary to identify the principal agents responsible for activity. Additionally, in vivo studies and toxicity evaluations are required to further validate the therapeutic applicability and safety of *A. desmettiana*. Nonetheless, the results of this study provide strong scientific evidence supporting the traditional use of *Agave* species and position *A. desmettiana* as a promising candidate for the development of novel antimicrobial agents, particularly against drug-resistant pathogens (Pandey et al., 2019).

CONCLUSION

The methanolic leaf extract of *Agave desmettiana* contains important bioactive phytochemicals and demonstrates significant antimicrobial activity against selected pathogenic and drug-resistant microorganisms. The low moisture and lipid contents, together with appreciable ash and elemental composition, suggest good stability and potential medicinal relevance. Overall, *A. desmettiana* represents a promising natural source of antimicrobial agents. Further studies focusing on isolation of active compounds, toxicity evaluation, and in vivo efficacy are recommended.

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