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Phytochemical Analysis of Dichrostachys Cinerea Leaves, Stem Bark, and Roots for Toothache Management

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Abstract
Toothache, a prevalent dental issue impairing productivity, stems from infections, decay, or inflammation, often inadequately managed by antibiotics due to resistance and flawed trials. This study explores the phytochemical potential of Dichrostachys cinerea (sickle bush), traditionally used in Nigeria for oral pain, to identify bioactive compounds for natural remedies. Leaves, stem bark, and roots were shade-dried, powdered, and sequentially extracted via Soxhlet with hexane, ethyl acetate, and methanol, yielding 1.06-10.70%. Preliminary screening detected saponins, tannins, flavonoids, alkaloids, and steroids in all extracts. Elemental analysis via sodium fusion confirmed carbon, hydrogen, nitrogen, sulfur, phosphorus, and chlorine. Thin layer chromatography separated fractions (Rf 0.50-0.90), followed by preparative isolation. Gas chromatography-mass spectrometry (7890B, 5978A) and infrared spectroscopy revealed a range of phytochemical constituents. Among the detected compounds were several whose mass spectra and fragmentation patterns showed similarity to known pharmacologically active molecules, such as those with anti-inflammatory (e.g., ibuprofen-like), sedative, and antimicrobial properties, based on library matching. However, the definitive biosynthetic origin and identity of these specific compounds as plant-derived metabolites require confirmation through isolation and advanced spectroscopic techniques (e.g., NMR). The collective phytochemical profile, including confirmed classes like flavonoids, tannins, and alkaloids, suggests a synergy of anti-inflammatory, antimicrobial, and antioxidant activities against pathogens like Staphylococcus aureus and Prevotella. This supports the plant's traditional use and indicates potential applications in herbal dentifrices and anti-inflammatory gels for sustainable toothache relief in resource-limited settings.
Keywords: Phytochemicals, Dichrostachys cinerea, toothache, antimicrobial, antioxidants

Introduction

Medicinal plants have been integral to healthcare systems worldwide, serving as vital sources of therapeutic agents. Their bioactive compounds offer valuable alternatives to synthetic drugs, particularly in regions with limited access to modern pharmaceuticals [1]. The global demand for herbal medicines underscores the need for systematic exploration and validation of traditional remedies [2, 3].

Many medicinal plants are rich in secondary metabolites such as tannins, terpenoids, alkaloids, and polyphenols, which exhibit potent antimicrobial properties. This is especially relevant in an era of growing antibiotic resistance [4]. These compounds can modulate oxidative stress and inflammation, supporting overall health [5]. However, a significant amount of traditional knowledge, including that related to dental care, remains undocumented and at risk of being lost [6].

Toothache, often resulting from dental infections, decay, or post-extraction complications, significantly impairs quality of life [11]. Common causative agents include polymicrobial communities with anaerobes like Prevotella and facultative anaerobes such as streptococci [7]. In some developing regions, conditions like dental fluorosis further exacerbate enamel defects and pain [12, 13]. The limitations of antibiotic treatments, including resistance and variable efficacy, highlight the necessity for alternative therapeutic strategies [7].

This study focuses on Dichrostachys cinerea (Fabaceae), a multipurpose tree known as sickle bush, which is abundant in the savannah regions of Nigeria. Ethnobotanical records document its traditional use for a wide range of ailments, including dysentery, headaches, and notably, toothaches [17, 18]. Previous phytochemical studies have reported the presence of alkaloids, tannins, flavonoids, and saponins in this species, while antibacterial activity against pathogens like Staphylococcus aureus and Escherichia coli has been confirmed [8, 16].

Given its traditional application for oral pain and documented bioactivity, D. cinerea represents a promising candidate for phytochemical investigation. This research aims to analyze the leaves, stem bark, and roots of D. cinerea to isolate and characterize their bioactive constituents. The goal is to provide a scientific basis for its traditional use in managing toothache and to evaluate

its potential as a source of anti-inflammatory, antimicrobial, and antioxidant agents for oral health.

Materials and Methods

Plant Description and Collection

Dichrostachys cinerea is a multipurpose tree with a fibrous bark and characteristic bi-colored, cylindrical flower spikes. Healthy leaves, stem bark, and roots were collected from the savannah habitat of Plateau State, Nigeria, in August 2023. A voucher specimen (FHJ768) was authenticated at the Herbarium of the Federal College of Forestry, Jos.

Preparation of Plant Extracts

The collected plant parts were shade-dried for two weeks and pulverized into a fine powder using a mechanical grinder. Approximately 50 g of each powdered sample (leaves, stem bark, roots) was subjected to sequential solvent extraction in a Soxhlet apparatus. Extraction was performed for 8 hours per solvent, starting with 250 mL of hexane, followed by ethyl acetate, and finally methanol. After each cycle, the solvent was removed using a rotary evaporator (Büchi R-300) at 40°C. The resulting crude extracts were weighed to calculate percentage yield and stored as aliquots at -20°C for further analysis.
Elemental Analysis (Sodium Fusion Test)

The presence of carbon, hydrogen, nitrogen, sulfur, phosphorus, and halogens was tested using Lassaigne's sodium fusion method. Briefly, a small pinch (approx. 0.05 g) of dried sodium metal was added to 0.05 g of each plant extract in a dry Pyrex test tube. The tube was heated gently until the sodium melted and reacted with the sample, then heated to redness. The hot tube was plunged into 10 mL of distilled water in a mortar and ground. The resulting mixture was boiled, filtered, and the filtrate (Lassaigne's filtrate) was used for specific tests as outlined in Table 1.

Phytochemical Screening

Standard qualitative chemical tests were conducted on the crude extracts to identify major classes of phytoconstituents, following established protocols [9,10].

Saponins: 1 g of extract was boiled with 10 mL of distilled water for 15 min. The mixture was cooled and shaken vigorously; persistent froth formation indicated saponins.

Tannins: 1 g of extract was dissolved in 10 mL of distilled water and filtered. A few drops of 5% ferric chloride solution were added to the filtrate; a blue-black coloration indicated tannins.

Flavonoids: 0.2 g of extract was dissolved in 2 mL of methanol and heated. A chip of magnesium ribbon and 2-3 drops of concentrated HCl were added. The appearance of a pink or orange-red color indicated flavonoids.

Alkaloids: 0.5 g of extract was warmed with 5 mL of 1% HCl on a water bath for 5 min and filtered. To 2 mL of the filtrate, 3 drops of Dragendorff's reagent were added; the formation of an orange-brown precipitate indicated alkaloids.

Steroids: 0.5 g of extract was dissolved in 3 mL of chloroform and filtered. Concentrated sulfuric acid (1 mL) was carefully added down the side of the test tube to form a lower layer; a reddish-brown ring at the interface indicated steroids.

Structural Determination

Fractionation and Isolation

The methanol extracts of leaves, stem bark, and roots were selected for fractionation based on preliminary phytochemical richness. Approximately 5 g of each crude extract was adsorbed onto silica gel (60-120 mesh) and loaded onto a glass column (50 cm x 3 cm) packed with silica gel (230-400 mesh). Separation was achieved using a stepwise gradient elution with increasing polarity: 100% hexane, followed by hexane:ethyl acetate mixtures (9:1, 3:1, 1:1, v/v), 100% ethyl acetate, ethyl acetate:methanol (1:1, v/v), and finally 100% methanol. A total of 300 mL of each solvent mixture was used. Fractions (50 mL each) were collected, monitored by analytical Thin Layer Chromatography (TLC), and combined based on similar TLC profiles.
Analytical TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck). The solvent system for development was ethyl acetate:methanol:water (40:5.4:5, v/v/v). Spots were visualized under UV light at 254 nm and 365 nm, and by spraying with vanillin-sulfuric acid reagent followed by heating. The retention factor (Rf) was calculated for each distinct band. Combined fractions were further purified using preparative TLC (silica gel GF_{254}, 1 mm thickness) with the same solvent system. Bands corresponding to single spots were scraped, eluted with methanol, filtered, and concentrated to obtain isolated compounds for instrumental analysis.

Instrumental Analysis

Gas Chromatography-Mass Spectrometry (GC-MS)

The isolated compounds and key fractions were analyzed using an Agilent 7890B Gas Chromatograph coupled with a 5978A Mass Selective Detector. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed: initial hold at 70°C for 2 min, then increased to 300°C at a rate of 10°C/min, with a final hold for 10 min. The injector temperature was set at 250°C in splitless mode. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The ion source temperature was 230°C, and the quadrupole temperature was 150°C. The mass scan range was 40-600 m/z. Compound identification was performed by comparing the mass spectra of the peaks with the data from the National Institute of Standards and Technology (NIST 17) library.

Fourier Transform Infrared (FTIR) Spectroscopy

Infrared spectra of the isolated compounds were recorded on a PerkinElmer Spectrum Two FTIR Spectrometer equipped with a Universal ATR (Attenuated Total Reflectance) sampling accessory. A small amount of each solid isolate was placed directly on the diamond ATR crystal and pressure was applied with

the anvil. Spectra were acquired over a wavenumber range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample. Background scans were collected before each sample. The spectra were analyzed to identify characteristic functional groups.

Results and Discussion

Percentage yield

The extraction yields varied significantly across plant parts and solvents, ranging from 1.06% for stem bark in ethyl acetate to 10.70% for leaves in hexane. This disparity reflects solvent polarity influencing the solubility of lipophilic versus polar metabolites; non-polar hexane favored extraction from lipid-rich leaves, while polar ethyl acetate yielded lower from fibrous bark [10]. Such yields are comparable to those reported for other Fabaceae species, where hexane extracts often exceed 8-12% due to terpenoid abundance [16]. These results underscore the efficiency of Soxhlet extraction for maximizing bioactive recovery, essential for therapeutic formulations targeting dental infections.

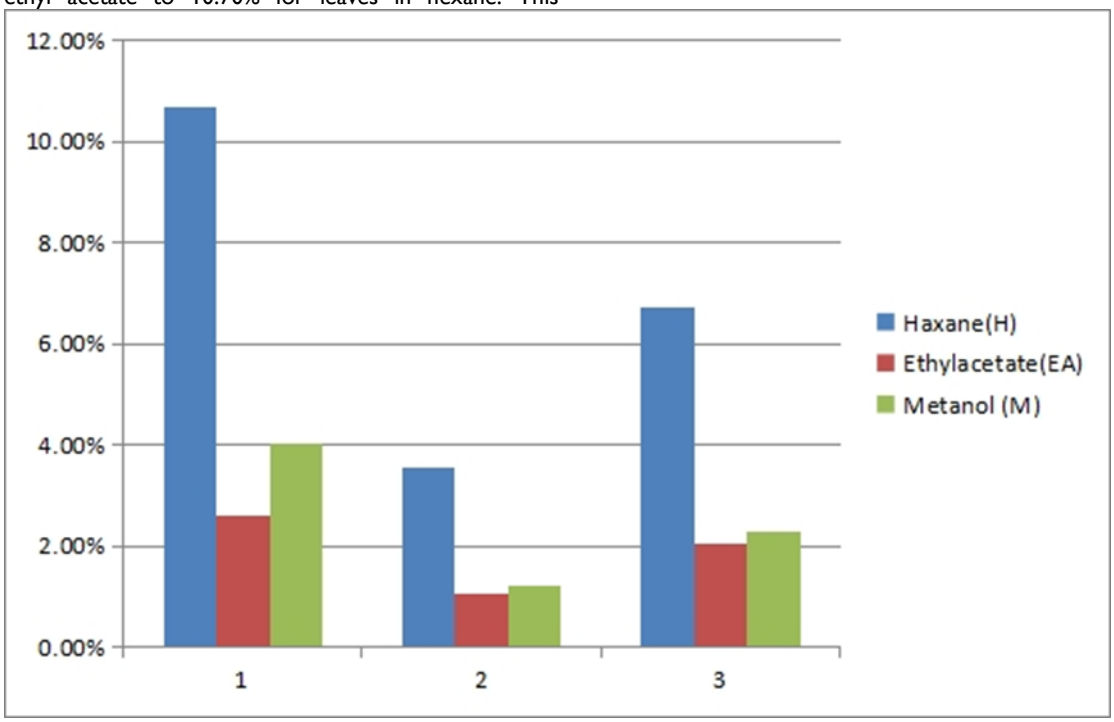

Figure 1: Percentage yields of extracts from leaves (1), stem bark (2), and roots (3) of D. cinerea

Preliminary elemental analysis

The sodium fusion test revealed a comprehensive elemental profile, confirming carbon, hydrogen, nitrogen, sulfur, phosphorus, and chlorine, with absence of fluoride. The CO2 evolution and milky lime water reaction affirm carbonaceous structures, while pink CoCl2 discoloration indicates hydrogen [9]. Nitrogen's brown prussian blue derivative and sulfur's black lead sulfide precipitate suggest alkaloid and thio-compound presence, pivotal for antimicrobial action against toothache pathogens like Staphylococcus aureus [19]. Phosphorus detection supports nucleoside-like metabolites, aligning with anti-inflammatory roles [5]. This profile validates D. cinerea as a reservoir for heteroatom-rich bioactives, enhancing its ethnopharmacological value [6].

Table 1: Preliminary elemental analysis

Test	Observation	Inference
Filtrate + CuO + heat	Gas evolved, turns milky with lime H_2O	CO_2 present
Droplet of liquid in (1) + $CuSO_4$	Pink color with $CoCl_2$ paper	Hydrogen present
Filtrate + HNO_3, boiled + $AgNO_3$(aq)	White ppt soluble in NH_4OH	Halogen (Cl)
Filtrate + glacial CH_3CO_2H + boiled	No ppt	CaF_2 absent
Filtrate + $FeSO_4$ + $FeCl_3$ + H_2SO_4	Brown coloration	N present
Filtrate + lead acetate + acetic acid	Black ppt	Lead sulfide present (S)
Mixture of Na_2CO_3 + KNO_3 + filtrate + heat	Yellow ppt	Phosphorus

Phytochemical screening

Qualitative screening demonstrated the ubiquitous presence of saponins, tannins, flavonoids, alkaloids, and steroids in all extracts, irrespective of solvent or plant part. This consistency highlights D. cinerea's biosynthetic robustness, with methanol extracts likely enriching polar phenolics like tannins for enhanced solubility [10]. Flavonoids and alkaloids, detected via magnesium-HCl and Dragendorff's tests, correlate with reported anti-plaque and analgesic effects in oral ethnomedicine [4,14]. Steroids' Liebermann-Burchard positivity suggests membrane-stabilizing potential against Fusobacterium-induced inflammation [7]. These metabolites synergistically combat polymicrobial dental biofilms, justifying traditional toothache applications and warranting quantitative assays for standardization [6,16].

Table 2: Results of phytochemical screening of D. cinerea extracts

Solvent	Phytochemical test	Leaves	Stem bark	Root
Hexane	Saponins	+	+	+
	Tannins	+	+	+
	Flavonoids	+	+	+
	Alkaloids	+	+	+
	Steroids	+	+	+
Ethyl acetate	Saponins	+	+	+
	Tannins	+	+	+
	Flavonoids	+	+	+
	Alkaloids	+	+	+
	Steroids	+	+	+
Methanol	Saponins	+	+	+
	Tannins	+	+	+
	Flavonoids	+	+	+
	Alkaloids	+	+	+
	Steroids	+	+	+

(+ = present).

Chromatography

TLC fractionation yielded six distinct profiles with Rf values from 0.50 to 0.90, enabling isolation of 10 fractions per plate. Leaves (Plate A) showed higher polarity bands (Rf 0.73-0.90), indicative of flavonoid glycosides, while stem bark (Plate B) exhibited moderate polarity (Rf 0.50-0.80), suiting terpenoid enrichment [10]. Preparative TLC confirmation of purity via single-spot visualization under UV (254 nm) ensures reliable downstream analysis, mirroring protocols for oral antimicrobial isolates [15]. These separations highlight solvent gradients' utility in resolving bioactive heterogeneity, crucial for targeted toothache therapeutics [16].

Table 3: Retention factor (Rf)

Annotated Rf	Corresponding bands	Rf calculated
Plate A (leaves)		
Rf1 (a-d)	4	0.81
Rf2 (e-g)	3	0.73
Rf3 (h-j)	4	0.90
Plate B (stem bark)		
Rf4 (a-c)	3	0.50
Rf5 (d-f)	4	0.80
Rf6 (g-j)	4	0.71

Instrumental Determination

Gas chromatography/mass spectrometry

NIST-matched GC/MS profiling tentatively identified 22 phyto-compounds across samples, with molecular weights spanning 102-414 Da and diverse formulas (e.g., $C_6H_{14}O$ to $C_{27}H_{44}O$). Leaves (Sample A) showed a higher prevalence of peaks with spectra similar to oxygenated terpenoids (e.g., cholecalciferol, MW 384), stem bark (Sample B) in peaks resembling phthalates (dibutyl phthalate, MW 278), and roots (Sample C) in peaks matching amino acids (histidine, MW 155) [16]. Retention times (3.5-10.7 min) correlated with volatility, propelled by He carrier gas in SIM mode for precise m/z fragmentation. The library matches suggest the presence of compounds with structural similarities to agents such as ibuprofen (anti-inflammatory) and monomyristin (antifungal), which could mechanistically disrupt Streptococcus anginosus biofilms and Prevotella enzymes [7,20]. However, these are tentative identifications requiring confirmation. Overlaps (e.g., ethyl octanoate in A and C) suggest conserved biosynthetic pathways, enhancing formulation potential [19].

Table 4: GC/MS data for Sample A

RT (min)	Mass data, m/z	Compound no.	MW	Molecular formula
3.5	102(100), 100, 148	1	102	$C_6H_{14}O$
4.2	134, 206(M+H), 134	2	206	$C_{10}H_{14}$
4.4	172(100), 186, 144	3	172	$C_{10}H_{20}O$
5.0	284(M+H), 384(100), 300, 223	4	384	$C_{27}H_{44}O$
6.1	414(M+H)(100), 358(M+H)	5	414	$C_{22}H_{38}O_7$
6.5	156.212(100), 288	6	212	$C_{10}H_{16}N_2O_3$
7.1	158(M+H), 164(M+H)(100)	7	164	$C_6H_{12}O_5$
8.8	179(M+H), 180, 180(100)	8	180	$C_9H_{12}N_2O_2$
10.0	302(100), 210, 296	9	302	$C_{17}H_{34}O_4$
10.2	271(M+H)(100), 192, 228	10	271	$C_{16}H_{17}O_3$

Table 5: GC/MS data for Sample B

RT (min)	Mass data, m/z	Compound no.	MW	Molecular formula
3.5	102(100)	11	102	$C_6H_{14}O$
4.4	142(100)	12	142	$C_7H_{10}O_3$
6.7	156, 156(100)	13	156	$C_{12}H_{12}$
8.0	166(100), 194	14	166	$C_{11}H_{10}N_2$
9.5	196(100), 211(M+H)	15	196	$C_{10}H_{12}O_4$
10.5	278(100)	16	278	$C_{16}H_{22}O_4$

Table 6: GC/MS data for Sample C

RT (min)	Mass data, m/z	Compound no.	MW	Molecular formula
4.4	172(100)	17	172	$C_{10}H_{12}O_2$
6.7	155(M+H)(100), 156	18	155	$C_6H_9N_3O_2$
7.6	212, 170(100)	19	170	$C_{13}H_{14}$
8.3	414(M+H)(100)	20	414	$C_{22}H_{38}O_7$
10.5	192, 103(M+H)(100)	21	103	$C_4H_9NO_2$
10.7	278(100)	22	278	$C_{16}H_{22}O_4$

Compound names:
3,3-Dimethylbutan-2-ol, Ibuprofen, Ethyl octanoate, Cholecalciferol, Ascorbic acid-6-palmitate, Butabarbital, Rhamnose, 7-Methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione, Monomyristin, Normorphine, 3,3-Dimethylbutan-2-ol, 2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone, Naphthalene-2,6-dimethyl, 2,4-Dihydro-1H-cyclopenta(b)-quinoxaline,1H,3H-Pyrano(3,4-C)pyran-5-carboxaldehyde, Dibutyl phthalate, Ethyl octanoate, Histidine, 5-Methyl-1-phenyl-hexa-1,3,5-triene, Ascorbic acid palmitate, D-2-Aminobutyric acid, Dibutyl phthalate.

Infrared spectroscopy
FT-IR spectra elucidated the functional group composition of the samples. Broad absorption bands in the 3500-2700 cm^{-1} region, present in all samples, are indicative of O-H and N-H stretches, characteristic of hydrogen-bonded phenolics, alcohols, and amines [10]. Sample A showed a distinct peak around 1782 cm^{-1}, corresponding to C=O stretching vibrations typical of esters or carboxylic acids, and C=C stretches indicative of alkenes. Sample B exhibited absorption in the 1800-1600 cm^{-1} range, consistent with conjugated C=O, C=N, and aromatic C=C bonds, suggesting the presence of nitrogenous aromatic systems. Sample C displayed a weak band in the 2500-2000 cm^{-1} region, which can be assigned to C≡N or C≡C functional groups. Absorptions in the fingerprint region (1400-600 cm^{-1}) confirmed the presence of C-O, C-N, and C-C bonds, consistent with glycosidic linkages and complex molecular frameworks [27]. The functional groups identified by FTIR (e.g., hydroxyls, carbonyls, aromatic systems) align with the major phytochemical classes (e.g., flavonoids, terpenoids, alkaloids) suggested by GC/MS and preliminary screening, supporting the extract's potential for multi-target biological activity relevant to oxidative stress and inflammation [4].

Table 7: IR data and spectrum interpretation

Band range (cm^{-1})	Groups	Sample A	Sample B	Sample C
3500-2700	N-H, O-H, C-H	O-H, C-H	O-H, N-H, C-H	O-H, C-H
2500-2000	C≡C, C≡N	-	-	C≡N
1800-1600	C=C, C=O, C=N	C=O, C=C	C=C, C=N	-
1400-600	C-C, C-O, C-N	C-C, C-O, C-N	C-C, C-O, C-N	C-C, C-O, C-N

Health benefits of compounds
The compounds tentatively identified via GC-MS library matching are reported in the literature to possess biological activities relevant to the symptoms and causes of toothache. For example:

Compounds with spectra similar to monomyristin are reported in literature to disrupt microbial cell membranes, exhibiting antimicrobial and antifungal activity [20].

Compounds matching butabarbital are documented as central nervous system depressants [21].

Compounds resembling cholecalciferol (vitamin D3) are known to support bone and immune health [22,13].

Compounds with profiles akin to ibuprofen are established nonsteroidal anti-inflammatory agents [23].

Compounds related to pyranopyran derivatives have been studied for antioxidant and antimicrobial properties [24].

Compounds similar to 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone are known flavoring agents [25].

Compounds matching histidine play a role in anti-inflammatory and metabolic processes [26].

Ascorbic acid derivatives are well-known for immune support and wound healing [5].

The collective presence of these compound classes, along with the confirmed phytochemicals (saponins, tannins, flavonoids, alkaloids, steroids) from preliminary screening, suggests a potential synergistic mechanism for managing toothache. This could theoretically involve antimicrobial action against oral pathogens, reduction of inflammation in pulpal and gingival tissues, and mitigation of oxidative stress mechanisms that are supported by the general bioactivity of the identified phytochemical classes [4, 19]. In vitro studies of other medicinal plants demonstrate that such principles can exert antimicrobial and antioxidant activities by targeting free radicals and disrupting pathogenic processes [27, 28, 29]. However, these proposed health benefits are inferred from literature on the pure compounds and the established bioactivity of the detected phytochemical classes. This inference provides a mechanistic hypothesis that aligns with the traditional analgesic and antiseptic use of D. cinerea [7, 16]. Direct experimental validation of antimicrobial, anti-inflammatory, or analgesic activity for these specific D. cinerea extracts is required to confirm these potential applications.

Conclusion

This study confirms Dichrostachys cinerea as a rich source of diverse bioactive compounds, with extraction yields of 1.06-10.70% and consistent detection of saponins, tannins, flavonoids, alkaloids, and steroids across plant parts. GC/MS and IR analyses tentatively identified 22 phyto-constituents, with spectral similarities to compounds associated with anti-inflammatory, antimicrobial, and antioxidant activities. Elemental profiling revealed a heteroatom-rich composition supportive of its therapeutic potential. The collective phytochemical profile provides a scientific basis for the traditional use of D. cinerea in managing toothache in Plateau-Nigeria, bridging ethnobotanical knowledge with modern phytochemical analysis. The findings position D. cinerea as a promising candidate for developing sustainable, plant-based alternatives for oral health. However, the tentative nature of the compound identifications and the lack of direct biological assays in this study highlight the need for future research. Subsequent work should focus on the isolation and unambiguous structural elucidation (e.g., via NMR) of the active principles, followed by in vitro and in vivo validation of antimicrobial, anti-inflammatory, and analgesic efficacy. Conservation efforts and standardization are also recommended to harness its full potential for clinical applications.

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