

Preliminary, chromatographic and spectrometric screening of the phytochemical properties of the phylla of *Spilanthes filicaulis* (Schumach & Thonn.) CD. AdamsAudu O. Isah¹, Habiba M. Muhammed², Mcdonald Idu³¹Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmaceutical Sciences, Federal University Lokoja, PMB 1154, Lokoja, Kogi State, Nigeria.²Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.³Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Edo State, Nigeria.**ABSTRACT**

Spilanthes. filicaulis belonging the family, Asteraceae, is known for its medicinal benefits and wide applications in the traditional treatment of varieties of ailments including toothache, malaria and typhoid fever especially in Nigeria. Several species within the genus *Spilanthes* are tropical plants that are extensively used in traditional medicine and as flavoring agents in food preparations. Although some individuals find the spilanthol-induced tingling sensation on the tongue unpleasant, cooking reduces this effect, allowing the plant to be consumed as a green leafy vegetable. Secondary metabolites are a valuable source of potential therapeutic agents. The plant is widely used in ethnomedicine, remains poorly validated scientifically. The current study investigated the phytochemical composition of *S. filicaulis* methanol extract. Qualitative phytochemical analysis was carried out using various chemical tests. The analysis revealed abundant alkaloids, moderate amount of saponins, flavonoids, and phenols, with minute quantity of glycosides, cardiac glycosides, and terpenoids, while tannins were absent. Gas Chromatography–Mass Spectrometry, a combination technique for separating and identifying compounds was carried out to check for the presence of specific compounds. Gas Chromatography–Mass Spectrometry (GC–MS) analysis further identified several compounds including benzyl alcohol, palmitoleic acid, dibutyl phthalate, n-hexadecanoic acid, and methyl stearate. The study shows that *S. filicaulis* contains bioactive compounds with perhaps promising pharmacological potential that may give credence to its traditional use in treating the aforementioned human health conditions. In addition, these findings suggest a lead to further exploration of the plant leaf as a potential source of novel therapeutic agents.

Keywords: Chromatography, Determination, phytochemical, Screening, Spectrometry, *Spilanthes filicaulis*

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Copyright: © 2026 Isah *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.**Introduction**

A myriad of medicinal plants including *S. filicaulis* have been exhaustively used traditionally for therapeutic purposes without regard for scientific validation of their bioactive constituents that maybe responsible for the pharmacological activities. This study sought after the secondary metabolites which may be responsible for its biological activities. Several species of the genus *Spilanthes* are found in the tropical parts of the world where they are used in traditional healing and food flavoring.¹ In culinary application, small amount of freshly shredded leaves impacts a unique flavor to salads, while both fresh and cooked leaves are also incorporated into stews and soups as delicacy in some parts of the World.^{1,2}

As opposed to synthetic compounds, natural products often display complex phytochemical scaffolds and unique pharmacological properties that enhance their therapeutic properties.^{2–4}

From time immemorial, plants have been playing a central role to traditional medicine, serving as remedies for a wide range of ailments and lead for many modern drugs.^{5–7}

Recently, there have been renewed interest in the plant kingdom as a sustainable and chemically diverse source of therapeutic molecules. Plant-derived compounds offer the possibility of novel modes of action and could play a pivotal role in addressing the global antibiotic resistance crisis^{8–11}. This makes natural products not only relevant but indispensable in the current search for a plethora of next-generation therapeutic agents. Amidst the numerous plants explored for antimicrobial activity, *S. filicaulis*, a plant native to Africa, stands out owing to its wide ethnomedicinal applications. Traditionally, it has been used to manage diverse health conditions, such as toothaches, respiratory tract infections, worm infection, cancers, infertility, poisoning, malaria and coughs.¹² Pharmacological studies have revealed that the species possesses cytotoxic, antioxidant, anti-inflammatory, and antidiabetic activities.^{13–15} The sub-acute oral administration of ethyl acetate extract of the species reportedly induced no significant biochemical abnormalities.¹⁶ Phytochemical screening reported the presence of bioactive compounds such as hexadecanoic acid and stigmasterol, isolated from combined hexane and methylene chloride extracts.¹² While crude extracts of *S. filicaulis* have demonstrated notable biological activity, relatively few isolated compounds have been characterized, and their specific antimicrobial roles remain largely unexplored.¹² Similarly, N-isobutyl-2E,4E,8E,10Z-dodeca-2,4,8,10-tetraenamide has been isolated from the aerial parts of *S. mauritiana*.² Other N-isobutylamides have also been reported in *S. alba*.¹⁷ These compounds collectively contribute to the distinct pungent taste and the bioactive potential of *Spilanthes* species, supporting their traditional applications in analgesic, antimicrobial, and anti-inflammatory therapies.

From the leaves of *S. ocyimifolia*, stigmasterol, the triterpenoid taraxasterol 7, and lupeyl acetate were isolated.¹⁸ A new triterpenoidal saponin olean-12-en-3-O- β -D-galactopyranosyl [1 $\rightarrow$ 4]-O- α -L-rhamnopyranoside was isolated from the roots of *S. acmella*. A

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steiractinolide derivative was isolated from *S. leiocarpa*.¹⁹ and eudesmanolide was isolated from the aerial parts of *S. leiocarpa*.¹ Bioassay-guided isolation from *S. acmella* resulted in the isolation of phenolics, vanillic acid 8, trans-ferulic acid 9, transisoferulic acid 10, coumarin, scopoletin 11, triterpenoid 3-acetylaleuritic acid 12, -sitostenone 13, stigmasterol, and stigmasteryl-3-O- β D-glucopyranoside, in addition to a mixture of stigmasterol and β sitosteryl-3-O- β D-glucopyranosides. *S. acmella* flower heads and *S. paniculata* have been reported to contain amino acids.¹

Supercritical fluid extraction (SFE) of *S. americana* showed that stems were particularly rich in sesquiterpenes (>40%), whereas leaves and flowers contained higher levels of nitrogenated (43% and 27%) and oxygenated compounds (36% and 23%). In *S. calva* DC., seven essential oil components have been identified, with caryophyllene oxide, caryophyllene, limonene, and myrcene being the dominant constituents.²⁰

Spilanthes products are widely marketed as over-the-counter herbal medicines and are frequently recommended by traditional healers in many countries. These products are derived from raw plant tissues or extracts and are commonly used for their analgesic, antibacterial, and antifungal properties.² Among the species, *S. acmella* is the most extensively studied due to its biological and pharmacological relevance, with most investigations focusing on its traditional medicinal applications. Because of the chemical variability among *Spilanthes* species, studies often highlight the main identified bioactive compounds to ensure accurate characterization and reproducibility.

In the current study, *S. filicaulis* leaf was subjected to preliminary phytochemical screening and GC-MS profiling to ascertain its potential bioactive constituents responsible for its plethora of biological activities.

Materials and Methods

Samples Collection

The whole plants of *Spilanthes filicaulis* were collected on the 14th

August, 2021 at Ajanaku village, Adavi Local Government Area, Kogi State, Nigeria and conveyed to the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. A plant taxonomist, Dr. H. A. Akinnibosun in the Department identified and authenticated it and the voucher specimen was prepared and deposited at the institutional Herbarium with the voucher number UBHdt/255 assigned for future reference.

Preparation of Plant Material

The fresh leaves were washed thoroughly 2-3 times with running tap water and once with distilled water, to remove adhering and extraneous materials. They were air-dried weight for two weeks to constant weight. The dried leaves were then blended using a household electric blender. The leaf powder was stored, sealed in labeled reagent bottles for later use.

Extraction of Plant Material

In each experimental procedure, about 10 g of the powdered plant material was weighed using a weighing balance and kept in a 200 mL glass container. About 100 mL of methanol was transferred to the powdered plant material. This was then shaken thoroughly and allowed to stay overnight. The solutions were then filtered and heated at 50°C until the solvent contents evaporated completely. The extracts were then collected, dried on water bath, weighed and the weight recorded.

Preliminary Phytochemical Assay

The confirmatory qualitative phytochemical screening of plant extracts were performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols and terpenoids) present in the extracts following standard protocols of²¹⁻²⁴ with some modifications.

Analysis of Phytochemical Compounds of *Spilanthes filicaulis* Leaf Extract

An analytical method of two techniques; Gas chromatography and mass spectrometry was used for separating and identifying individual phytochemical compounds which are abundant in the leaf of *S. filicaulis*. In GC technique, gas was injected and pushed into an inert carrier through a long, coiled capillary column. The column slowly separates the chemical mixture over time (retention time). In the MS technique, as compounds exited the GC column, they were bombarded with electrons to break them into charged fragments. These fragments travelled through a magnetic field and counted. The software in the system plotted these data as chromatograms showing the abundance of compounds over time, paired with mass-to-charge ratios for precise.

The gas chromatography-mass spectrophotometer (GC-MS) analysis of the extracts was carried using a gas chromatography (Agilent 7820 A) coupled to 5975 C inert mass spectrometer GC-MS (with triple-axis detector) with an electron impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% phenyl methyl siloxane (30 m length x 0.32 mm diameter x 0.25 μ m film thickness). Helium was the carrier gas used at a ramped flow of 1.5 mL/min (0 min), 9.9 mL/min to 2 mL/min (0.35 min), 10 mL/min to 1.2 mL/min (0.05 min) at an initial nominal pressure of 1.56 psi and average velocity of 44.41 cm/s. 1.0 μ L of the samples were injected in splitless mode at an injection temperature of 250°C. Purge flow to split vent was 30.0 mL/min at 0.35 min with a total flow of 31.94 mL/min; gas saver mode was turned off. Initially, the oven was programmed at 40°C (1 min) then ramped at 15°C/min to 100°C (3 min) then, followed by 20°C/min to 240°C (5 min) and finally, 10°C/min to 310°C (8 min).

The mass spectrophotometer (MS) was operated in scan/SIM mode (35–500 amu) with electron impact ionization (EI) mode at 70 eV with ion source temperature of 230°C, a quadrupole temperature of 150°C and transferred line temperature of 300°C. Before analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using the established criteria to check the abundance of m/z (mass/charge ratio) 69, 219, 502 and other instruments optimal and sensitivity conditions. Compound identification was performed by comparing mass spectra with the National Institute of Standards and Technology (NIST) library^{25, 26}.

Results and Discussion

The table 1 below shows the presence of different classes of phytochemicals in the sample. Alkaloids, saponins, flavonoids, and phenols, glycosides, cardiac glycosides, and terpenoids are present (+), while tannins are absent (-).

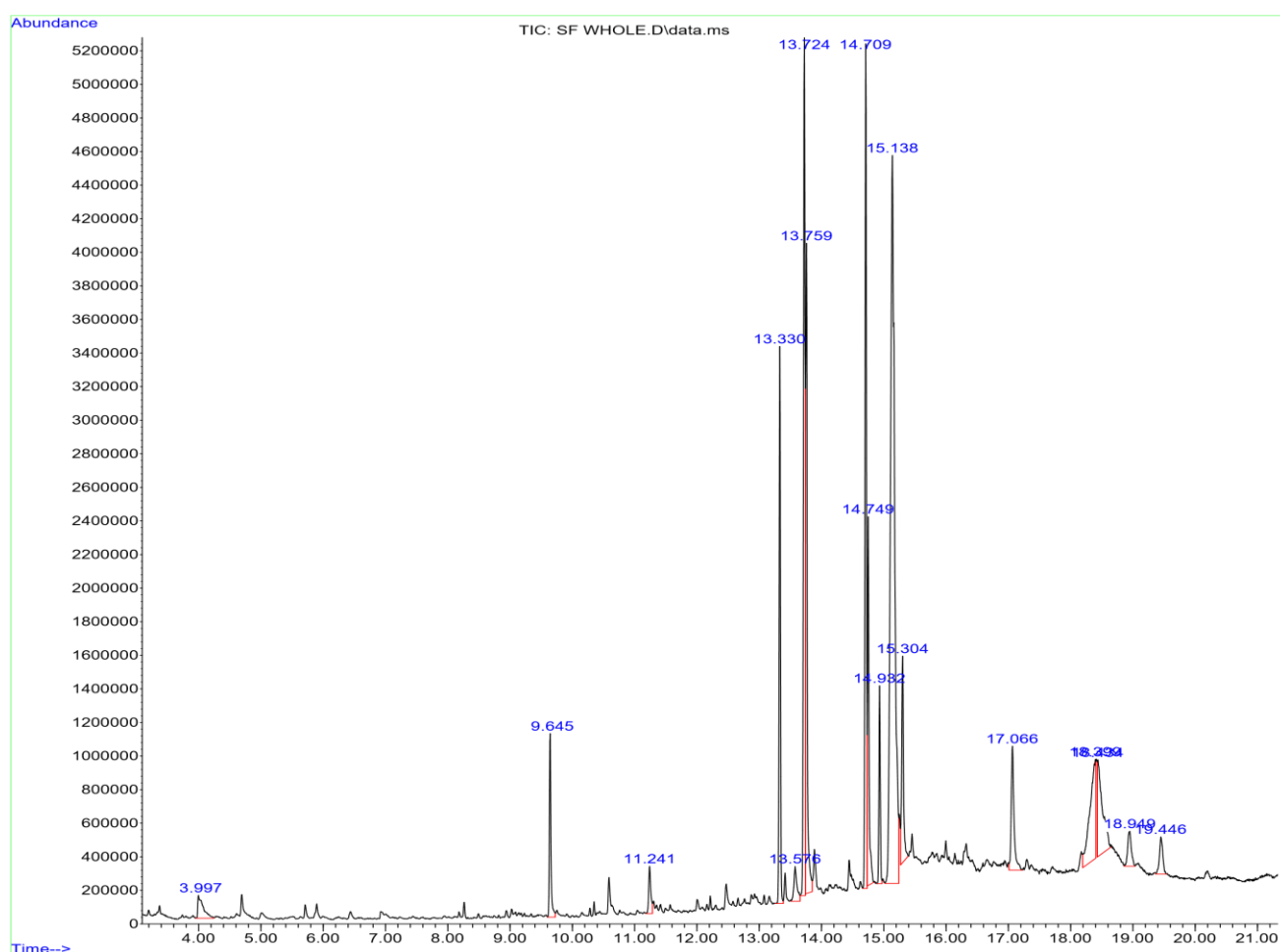
The phytochemical profile of *S. filicaulis* leaf, revealed abundant presence of alkaloids, moderate amount of saponins, flavonoids, and phenols, with low quantity of glycosides, cardiac glycosides, and terpenoids, while tannins are found absent (Table 1). This aligns with reported findings indicating that *Spilanthes* species, including *S. filicaulis*, contain diverse bioactive compounds such as alkaloids and flavonoids, that may be responsible for their pharmacological effects.¹ Alkaloids like spilanthal, predominantly found in *Spilanthes* leaves, are known for their analgesic and immunomodulatory activities. The moderate levels of flavonoids and phenols support the plant's antioxidant properties reported in some literatures, as these compounds are effective free radical scavengers and protectors against oxidative stress associated with various diseases.²⁶ Saponins may contribute to anti-inflammatory and antimicrobial effects. The low presence of glycosides and cardiac glycosides might suggest minor contributions to heart-related benefits, while terpenoids at low levels are consistent with known terpenoid constituents with anti-inflammatory effects in *Spilanthes filicalis*. The absence of tannins is also documented in some studies on *S. filicaulis* and suggests limited astringent activity that may be observed if tested for.²⁷ In addition, the phytochemical composition of *S. filicaulis* leaf supports its traditional use in herbal medicine. The therapeutic activity against some health conditions may be attributed primarily to alkaloids, flavonoids, and phenolic compounds.

Table 1: Results of the preliminary phytochemical screening of *Spilanthes filicaulis* leaf

S/N	Phytochemicals	Results
1	Alkaloid	+
2	Glycoside	+
3	Saponins	+
4	Tannins	-
5	Cardiac glycoside	+
6	Flavonoid	+
7	Phenols	+
8	Terpenoids	+

Key: + Presence of components, - Absence of components

GC-MS analysis as partly depicted in figure 1 showed the presence of bioactive phytochemical compounds that are likely responsible for antibacterial and other biological activities.

**Figure 1:** A Sample of the Chromatograms generated from the GC-MS analysis

For instance, Benzyl alcohol (Table 2) acts as an antimicrobial by disrupting microbial cell membranes and inhibiting bacterial and fungal growth. It is widely used as a preservative in pharmaceutical formulations due to its ability to inhibit a broad spectrum of microorganisms, including bacteria and fungi. Its local anesthetic effect could also help in reducing infection-related pain. 2,2,4-Di-tert-butylphenol (Table 2) has strong antimicrobial and antifungal effects, targeting resistant strains by disrupting cell walls and biofilm formation. It exhibits significant antioxidant activity that helps protect cells from oxidative damage during infection. Its synergistic effect with other antimicrobial agents makes it a promising compound for

overcoming microbial resistance. 4-Hydroxy-beta-ionone (Table 2), A carotenoid-related compound with antimicrobial effects achieved by inhibiting microbial enzymes and growth regulators. Its antioxidant activity also supports host defense mechanisms, enhancing overall antimicrobial efficacy.

Hexadecanoic acid, methyl ester (Methyl palmitate) shows antimicrobial activity by interfering with microbial membrane integrity and function. This compound also has anti-inflammatory properties that reduce infection-induced inflammation, aiding in faster recovery. n-Hexadecanoic acid (Palmitic acid) (Table 2), Palmitic acid acts against a range of bacteria and fungi by disrupting lipid

bilayers. It also modulates immune responses, enhancing the body's natural antimicrobial defenses. The antimicrobial activity of *S. filicaulis* extracts may be attributed to the presence of identified phytochemicals. To support this, n-hexadecanoic acid isolated from *Ipomoea eriocarpa* leaves has been shown to possess antioxidant activity and inhibit *S. aureus*, *B. subtilis* and *E. coli* ²⁸.

9,12-Octadecadienoic acid, methyl ester (Methyl linoleate) (Table 2). Its antimicrobial activity comes from its ability to alter cell membrane fluidity and function in pathogens. It also demonstrates anti-inflammatory effects that complement its antimicrobial role, especially in wound healing. 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate). This compound exhibits antimicrobial effects against Gram-positive and Gram-negative bacteria. It may impede microbial adherence and biofilm formation, important factors in pathogenicity and resistance. Methyl stearate has been reported to inhibit bacterial proliferation, possibly by disrupting membrane transport mechanisms. Additionally, it provides neuroprotective benefits that could support infectious disease management where neural damage occurs. 9,12-Octadecadienoic acid (Linoleic acid) (Table 2), One of the essential fatty acids known to inhibit a wide variety of bacteria and fungi, partly through damaging cellular membranes and interfering with signal transduction in pathogens. It can also enhance antifungal drug effectiveness in combination treatments. Similarly, 9,12-octadecadienoic acid, a polyunsaturated fatty acid, has been detected in medicinal some plant extracts exhibiting significant antimicrobial effects. ^{29,30} Furthermore, phytosterols for instance, sitosterol and stigmasterol have also previously been reported to demonstrate antimicrobial activities ³⁰.

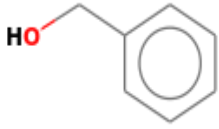
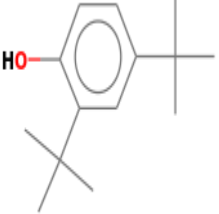
Hexadecenoic acid, 2-hydroxy- (Hydroxy palmitic acid) (Table 2) possesses broad-spectrum antimicrobial activities targeting bacteria and fungi by disrupting membrane stability and critical enzyme systems. Its antioxidative and anti-inflammatory properties also mitigate tissue damage during infections. Worthy of note was that Hexadecenoic acid has been previously isolated in pure form from a combination of hexane and methylene chloride extracts of *S. filicaulis* whole plant. ³¹

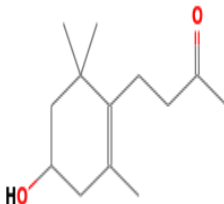
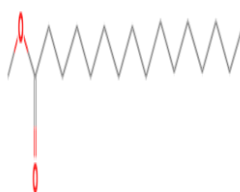
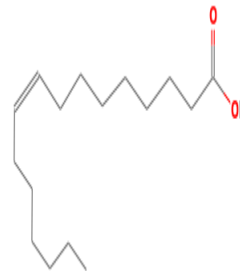
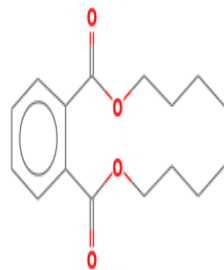
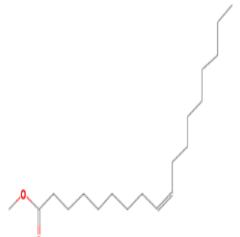
Other compounds of significant effects are: Palmitoleic acid, a monounsaturated fatty acid that exhibits antioxidative and antidiabetic effects. It improves insulin sensitivity and reduces chronic inflammation, helping in metabolic disorders. It also plays a role in lipid metabolism and cardiovascular health. Dibutylphthalate (Table 2) is primarily an industrial plasticizer with no clear medicinal benefits. It is best known for potential toxicity and endocrine-disrupting effects, thus typically avoided in therapeutic formulations.

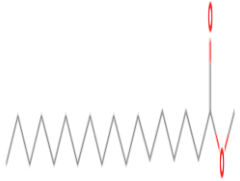
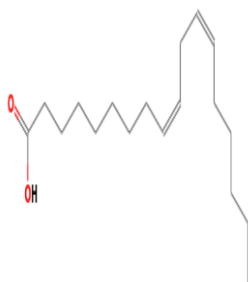
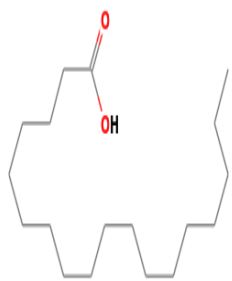
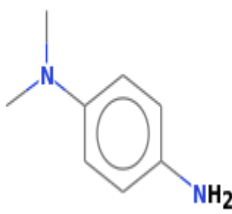
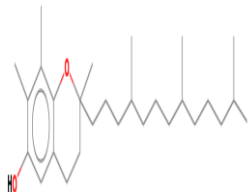
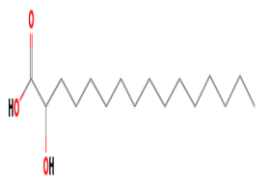
9-Octadecenoic acid, known as a monounsaturated fatty acid methyl ester, it acts as an antioxidant and anticancer agent by regulating apoptosis and oxidative stress pathways. Octadecanoic acid (Stearic acid), (Table 2) a saturated fatty acid with moisturizing, anti-inflammatory, and anti-aging properties, often used in skincare formulations to improve skin barrier function. 1,4-Benzenediamine, N-(1,3-dimethylbutyl), (Table 2), an aromatic amine with antioxidant and cardioprotective actions. It may prevent apoptosis and cellular damage in cardiovascular tissues by modulating oxidative stress pathways. Gamma-Tocopherol (Vitamin variant) (Table 2) A form of vitamin E with potent antioxidant, anticancer, anti-inflammatory, and cardioprotective effects. It neutralizes reactive oxygen species, stabilizes membranes, and modulates immune functions. Dibutyl phthalate (Table 2) has no therapeutic benefit, but mainly noted for industrial use and is of toxicity concerns.

It is noteworthy that phytochemical compounds such as n-hexadecanoic acid and 9,12-octadecadecenoic acid are reported by ³² and the current study from the phytochemical profiling of *S. filicaulis* using same whole plant sample, solvent and GC-MS technique. However, stigmasterol, sitosterol, phytol, 2,4-decadienal and 13-docosenamide which are reported in ³² were not detected in the current investigation of the same plant species. Similarly, Benzyl alcohol, 2,2,4-Di-tert-butylphenol, 4-Hydroxy-beta-ionone and Dibutylphthalate are detected in the current study but not reported in ³². These variations in the compositions of secondary metabolites could be due to geographical and climatic conditions, altitude and elevation, soil compositions and nutrients, genetic variants, developmental and phenological stage, and perhaps, the time and season of harvest.

Table 2: Phytochemical compounds obtained from GC-MS analysis of *Spilanthes filicaulis* leaf

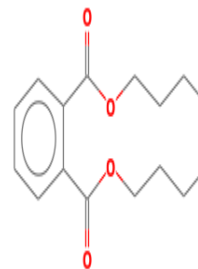
S/No	RT (Min)	Phytochemical compound IUPAC NAME	Molecular weight	Peak % Area	Molecular formula	Molecular structure	Medicinal properties
1	3.997	Benzyl alcohol	108.1378	1.14	C ₇ H ₈ O		Anaesthetic, Antiparastic
2	9.645	2,4-Di-tert-butylphenol	206.3239	2.62	C ₁₄ H ₂₂ O		Antioxidant, antiinflammatory, antimicrobial, anticancer

3	11.241	4-Hydroxy-.beta.-ionone	210.3126	0.94	C ₁₃ H ₂₂ O ₂		Antioxidant, antiinflammatory, antimicrobial, anticancer
4	13.330	Hexadecenoic acid, methyl ester	270.4507	6.65	C ₁₇ H ₃₄ O ₂		Antioxidant, antihyperglycaemic, antiarthritis, hepatoprotective, anticancer, antiinflammatory
5	13.576	Palmitoleic acid	254.4082	0.99	C ₁₆ H ₃₀ O ₂		Antyidiabetic, antioxidants
6	13.724	Dibutyl phthalate	278.3435	13.31	C ₁₆ H ₂₂ O ₄		No known benefit
7	13.759	n-Hexadecenoic acid	256.4241	8.79	C ₁₆ H ₃₂ O ₂		Antioxidant, antiinflammatory, antimicrobial, anticancer
8	14.709	9,12-Octadecadienoic acid, methyl ester.	294.4721	11.10	C ₁₉ H ₃₄ O ₂		Anti-inflammatory, anti-arthritis, antimicrobial
9	14.749	9-Octadecenoic acid (Z)-, methyl ester	296.4879	4.85	C ₁₉ H ₃₆ O ₂		Antioxidants, anticancer

10	14.932	Methyl stearate	298.5038	2.40	C ₁₉ H ₃₈ O ₂		Antimicrobial, neuroprotective against cardiac arrest
11	15.139	9,12-Octadecadienoic acid	280.4455	28.60	C ₁₈ H ₃₂ O ₂		Antioxidant, antiinflammatory, antimicrobial, anticancer
12	15.304	Octadecanoic acid	284.4772	3.47	C ₁₈ H ₃₆ O ₂		Moisturizing, anti- inflammatory, anti- aging
13	17.066	1,4-Benzenediamine, N-(1,3-dime	136.1943	3.18	C ₈ H ₁₂ N ₂		Antioxidants, cardioprotective, antiapopto
14	18.399	gamma.-Tocopherol	416.6795	5.44	C ₂₈ H ₄₈ O ₂		Anticancer, antiinflammatory, cardioprotective
15	18.149	Hexadecanoic acid, 2-hydroxy-	272.4235	1.18	C ₁₆ H ₃₂ O ₃		Antioxidant, antiinflammatory, antimicrobial, anticancer, antiulcer, antimalarial

16 19.446 Dibutyl phthalate 278.3435 1.14 C₁₆H₂₂O₄

No known



Conclusion

The findings in the current study contribute to broadening of knowledge of plant-derived secondary metabolites and provide some leads for future drug discovery and development. However, the revealed bioactive compounds in *S. filicaulis* leaf need to be subjected to pharmacological investigations on *in vitro* and *in vivo* studies, as well as toxicity profiling, and clinical trials to essentially ascertain their pharmacological effects, safety and therapeutic efficacy on some ailments. Also, further research is required to investigate the synergistic use of these compounds or with conventional therapeutic agents to overcome resistance.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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