



Phytochemical characterization and cytotoxicity effects of *Hyptis suaveolens* collected in Nigeria

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ABSTRACT

Hyptis suaveolens (L.) Poit. belongs to the *Lamiaceae* family. This weed, native to tropical America, has become widespread in tropical regions worldwide. In folk medicine, plant extracts are used in the treatment of wounds, peptic ulcers, respiratory tract infections, and skin diseases among others. Thus, the importance of assessing the toxicity of plants for any human use cannot be overstated. This study analyzed the phytochemical properties of the Nigerian *H. suaveolens* population and assessed its cytotoxicity. Six bioactive compounds were isolated from the acetone extract of the *H. suaveolens* plant using silica gel column chromatography, one of which is new from natural sources. The isolated compounds were identified as 14-*O*-acetyl suaveolol (**C1**), suaveolol (**C2**), suaveolic acid (**C3**), dehydroabietinol (**C4**), maniladiol (**C5**), and β -sitosterol (**C6**) based on 1D and 2D NMR spectroscopic data. The MTT assay was used to assess the cytotoxicity effects of these metabolites on HepG2, KMST-6, and HaCaT cells. Among them, **C1** significantly reduced HepG2, KMST-6, and HaCaT cell viability with IC₅₀ values of 35.97, 43.48, and 65.10 μ g/ml, respectively. The study suggests **C1** has promising anticancer properties, possibly due to the acetyl group on C-14, while **C2**, suaveolol, lacks this group, and did not yield any inhibitory activities. However, further research is needed to understand its mechanisms.

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1. Introduction

Hyptis suaveolens (L.) Poit. also known as “bush mint” in tropical America, has become a widespread weed in tropical regions worldwide, including Nigeria (Chukwujekwu et al., 2005). The plant's adaptability and survival are facilitated by its diverse composition, which allows it to thrive in various environmental conditions. *H. suaveolens* is one of the most common members of Lamiaceae family which in traditional medicine is believed to have insecticidal and repellent properties against several insect species (Tripathi et al., 2013; Ngamo et al., 2007). In West Africa, the leaves of the plant are

traditionally used by farmers to protect cowpea seeds against bruchids damage (Sanon et al., 2006). Additionally, the plant's ethnopharmacological significance is evident in various countries, such as India, where seeds are used for gynecological disorders and leaf extracts for snake bites and skin diseases (Bhargava et al., 2020; Vidyasagar and Prashantkumar, 2007). In Brazil, the whole plants' infusion is utilized to treat diarrhoea, digestive system, and headache (Gonçalves and Pasa, 2015). In Nigeria, the whole plant juice and dried leaf smoke are used as mosquito repellent (Salawu et al., 2021). Other uses include the treatment of cold and coughs (Sourabié et al., 2013). Several phytochemicals have been identified from various parts of the plant, including quercetin-3-*O*- β -d-glucopyranoside, apigenin-6,8-di-*C*-glucoside, kaempferol-3-*O*-(4-*O*-acetyl- α -l-rhamnopyranoside), apigenin, sorbifolin, quercetin, kaempferol, genkwanin, rutin, rosmarinic acid, methyl rosmarinic acid, ursolic acid, caffeic acid, gallic acid, and adenosine (Hsu et al., 2023; Tang et al., 2019). These metabolites are known to have significant biological effects such as

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anti-inflammatory (Tian et al., 2021; Hong et al., 2021), anticancer (Singh et al., 2023; Ezzati et al., 2020), antidiabetic (Miao et al., 2023), antioxidant (Ogunro et al., 2022; Shokoohinia et al., 2015), and antibacterial (Kim et al., 2020; Karpiński et al., 2019) properties. The importance of assessing the toxicity of plants for any human use cannot be overstated. Thus, this study aims to analyze the phytochemical constituents of the Nigerian *H. suaveolens* population and assess its cytotoxicity.

2. Materials and methods

2.1. Equipment and reagents

The 1D and 2D NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer (Bruker, Rheinstetten, Germany) in CDCl₃ with TMS as internal reference. Column chromatography was performed using normal-phase silica gel 60 (70–230-mesh ASTM; Merck, Kenilworth, NJ, USA). The fractions were monitored using thin layer chromatography (TLC) on silica gel aluminum sheets (Silica gel 60 F254; Merck). A vanillin sulfuric acid reagent (10 % H₂SO₄) and heating to 105. °C were used for detection of the spots. The study utilized high-quality chemicals and reagents: Trypan Blue (TB), penicillin, streptomycin, Millex syringe filter units (0.22 μm, 0.45 μm), and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) were ordered from Sigma-Aldrich (Steinheim, Germany). Phosphate buffered saline (PBS) with Ca²⁺/Mg²⁺ was purchased from Oxoid Limited (England). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin/ethyl diamine tetra acetic acid (EDTA) (0.25 %) were bought from Gibco Invitrogen (Germany). While tissue culture plates (6-, 24- and 96-well plates) were purchased from Greiner Bio-One (Germany).

2.2. Plant collection and extraction

Hyptis suaveolens (L.) was collected from Saki, Oyo State, South-west Nigeria and identified by a botanist Mr O Samson. A voucher specimen (No. TOPSH023045) is deposited at the Oke-Ogun Polytechnic Saki Herbarium. The leaves were harvested during the vegetative stage for optimal bioactive compounds, air-dried in the shade for 48 h, and powdered using IKA® MF 10.2 impact grinder head (1.00 Kg). Extraction was performed using acetone (2 × 2 L) at a solvent-to-plant ratio of 2:1 via maceration for 48 h. The extract was filtered and concentrated with a Buchi® cold-trap condenser, yielding 34.0 g.

2.3. Isolation of the phytochemicals

The crude *Hyptis suaveolens* (H.S) extract was fractionated using a gradient elution system of hexane: ethyl acetate (100: 0 → 0: 100 %) followed by ethyl acetate: methanol (100: 0 → 80: 20 %) on silica gel column chromatography, similar fractions were combined based to their similarity on the TLC to give the main fractions (F_{I-XL}). C1 and C2 were obtained independently after purifying fractions four (F_{IV}) and ten (F_X) of the main columns using a hexane: ethyl acetate gradient. While C3 and C6 were obtained directly from fractions three (F_{IX}) and nine (F_{III}) of the main column, respectively. C4 was obtained through isocratic elution of main fraction twenty-three (F_{XXIII}) with hexane: ethyl acetate (9:1). While C5 was obtained through preparative chromatography of subfraction twelve (F_{V-12}) after re-chromatography of fraction five (F_V) using a hexane: ethyl acetate gradient.

2.4. Physical properties of C1

White powder (173 mg). ¹H (Table 1, Figure S1 400 MHz, CDCl₃), ¹³C (Table 1, Figure S1 100 MHz, CDCl₃), Figure S3: DEPT-135 (CDCl₃), Figure S4: COSY (CDCl₃), Figure S5: HSQC (400 MHz, DMSO-d₆),

Table 1
¹H (400 MHz) and ¹³C NMR (100 MHz) data of compound 1 (C1) in CDCl₃.

Position	δ _H (mult., J in Hz)	δ _C	HMBC
1	1.43 and 0.81 (m)	35.5 CH ₂	C-20
2	1.30 (m)	18.1 CH ₂	C-4, C-10
3	1.13 and 1.01 (m)	34.8 CH ₂	C-4, C-18
4	—	37.4 C	—
5	1.19 (m)	44.5 CH	—
6	1.42 and 0.90 (m)	21.5 CH ₂	—
7	1.77 and 1.65 (m)	24.0 CH ₂	—
8	—	125.8 C	—
9	—	144.3 C	—
10	—	37.7 C	—
11	1.75 and 1.53 (m)	27.3 CH ₂	C-8, C-9
12	1.18 (m)	18.0 CH ₂	C-9
13	1.21 (m)	45.2 CH	—
14	5.11 (d, 8.8)	75.5 CH	OCCH ₃ , C-9, C-8, C-13, C-15
15	1.34 (m)	26.8 CH	C-13
16	0.64 (d, 7.0)	20.8 CH ₃	C-13, C-15, C-17
17	0.53 (brd, 7.0)	16.8 CH ₃	C-13, C-15, C-16
18	3.13 (d, 10.8)	71.7 CH ₂	C-3, C-4, C-5, C18
	2.86 (d, 10.8)	—	C-3, C-4, C-5, C18
19	0.50 (s)	17.4 CH ₃	C-3, C-4, C-5, C-19
20	0.72 (s)	19.5 CH ₃	C-1, C-5, C-9, C-10
OCCH ₃	—	171.7 C	—
OCCH ₃	1.78 (s)	21.1 CH ₃	COCH ₃

Figure S6: HMBC (400 MHz, DMSO-d₆), Figure S7: HRESIMS, *m/z* = 289.2712 (99 %) [M – 59]⁺ (calculated for C₂₀H₃₃O, 289.25).

2.5. Cell viability on MTT assay

2.5.1. Cell line and cell culture

HepG2, KMST-6, and HaCaT cell lines were the selected choice in this study (Manassas, USA). They were cultivated at 37 °C in 95 % air and 5 % CO₂, after standard aseptic work procedures. The cells were separately cultured in 75 cm² culture flasks using full DMEM growth media containing 10 % fetal bovine serum, 1 % penicillin (100 IU/ml), and streptomycin (100 μg/ml).

2.5.2. Cytotoxicity assay

Cytotoxicity was assessed using MTT. HepG2, KMST-6, and HaCaT cell lines were individually plated in 96-well cell culture plates with a cell density of 5000 cells per well. The plates were then incubated for a whole night to facilitate adhesion. Following this, the medium was replaced with varied concentrations (12.5, 25, 50, 100, and 200 μg/ml) of the tested metabolites (1–6) and H.S extract. Cells were incubated with medium for 24 h, then added with MTT solution (10 μL of 5mg/ml) and incubated for another 4 h at 37 °C. MTT crystals were solubilized using DMSO, absorbance read (570 nm), and percentage cell viability calculated.

$$\text{Cell viability} = \frac{\text{Absorbance of treated well}}{\text{Absorbance of untreated well}} \times 100$$

2.5.3. Analytical statistics

This study used GraphPad Prism for Windows (GraphPad Software, San Diego, US; version 8.4.3) to record and analyze data, with triplicate runs. Results were deemed statistically significant when *p* < 0.05, confirming the Kolmogorov-Smirnov test for normal distribution.

3. Results and discussions

3.1. Chemical characterization

Fractionation of the crude extract from *H. suaveolens* through repeated column chromatography on silica gel and/ or Sephadex LH-

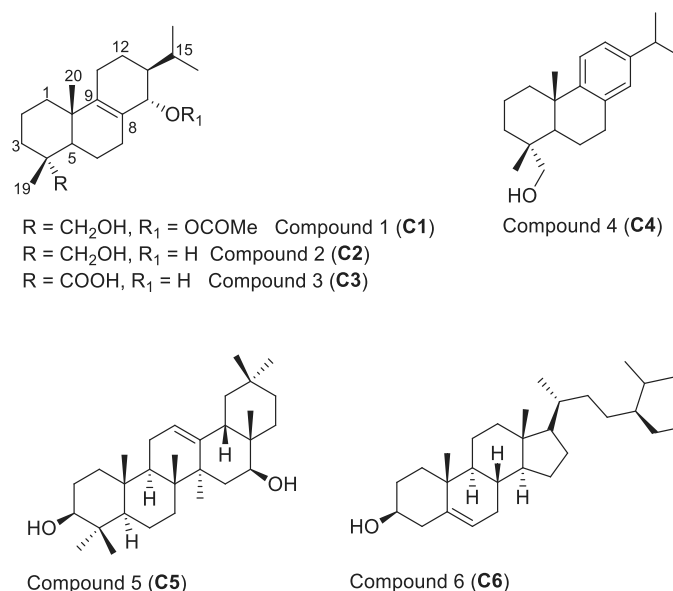


Fig. 1. Isolated compounds (**C1–C6**) from the leaves of *Hyptis suaveolens*.

20 yielded 5 known compounds and one new. The chemical characterization of the compounds was achieved using 1D (¹H, ¹³C, and DEPT-135) and 2D-NMR experiments (COSY, HSQC, and HMBC), FTIR, and MS data, as well as corroboration with existing data (Fig. 1).

C1 was isolated as a white powder. Careful inspection of the proton (¹H) NMR (Table 1, Supplementary Material) revealed a prominent signal appearing at δ_H 5.11 (*d*, *J* = 8.8 Hz, H-14), which was indicative of an oxygenated methine. Furthermore, four distinct methyl signals were detected as sharp singlets at δ_H 0.72 (*s*, H-20), 0.64 (*d*, *J* = 7.0 Hz, H-16), 0.53 (*d*, *J* = 6.9 Hz, H-17), 0.50 (*s*, H-18). Additionally, a methyl signal corresponding to an acetyl group was observed at δ_H 1.78 (Oaikhena et al., 2024; Islam et al., 2014; Supplementary Material Figure S1). Further observation was made for the methylene signals at δ_H 3.13 (*d*, *J* = 10.8 Hz, H-18_b) and 2.86 (*d*, *J* = 10.8 Hz, H-18_a), which are characteristic to protons in proximity to a primary hydroxyl group (Vera-Arzave et al., 2012; Grassi et al., 2006). Several overlapped or partially overlapped peaks were observed as clusters between δ_H 1.77–0.81 ppm. The above data indicate an abietane skeleton (Cao et al., 2019; Etsassala et al., 2019; Hussein et al., 2007). The ¹³C NMR data (Table 1) showed twenty (20) carbon signals, which supported the diterpene nature of the compound. DEPT-135 NMR experiment was used to differentiate between the ¹³C signals. Thus, they were categorically identified as four quaternary (δ_C 144.3, 125.5, 37.7, and 37.4), four methyls (δ_C 20.8, 19.5, 17.4, and 16.8), eight methylenes (δ_C 71.7, 35.5, 34.8, 27.3, 24.0, 21.5, 18.1, and 18.0), and four methines (δ_C 75.5, 45.2, 44.5, and 26.8). Furthermore, two additional signals were observed at δ_C 171.7 and 21.1, which were attributed to an acetyl group. The 1D and 2D NMR data analysis unambiguously identified **C1** as the acetylated derivative of the known compound suaveolol, which is a new finding. The connectivity of the acetyl group on C-14 was corroborated through long range proton-carbon correlation of H-14 with COCH₃ (δ_C 171.7) as shown in Fig. 2. In addition, HMBC showed correlations of H-14 with C-8 (125.5), C-9 (144.3), C-13 (45.2), and C-15 (26.8) to further support this assignment. The C-14 configuration is assigned as depicted in Fig. 2 based on NMR data similarity with the parent structure and biogenetic basis, as the parent compound and **C1** were isolated from the same source. Therefore, based on the spectroscopic evidence, **C1** is hereby identified as an acetylated derivative of the known compound suaveolol. This is the first report of the compound from natural sources and is hereby given the 14-*O*-acetyl suaveolol or suaveolol acetate.

The known compounds (**C2–C6**) were compared to the previously published data, and identified as suaveolol (**C2**) (Oaikhena et al., 2024), suaveolic acid (**C3**) (Oaikhena et al., 2024; Islam et al., 2014), dehydroabietinol (**C4**) (Oaikhena et al., 2024), maniladiol (**C5**) (Moraes et al., 2024; Sokoudjou et al., 2020), and β -sitosterol (**C6**) (Uttu et al., 2023; Astuti et al., 2022; Anwar et al., 2020), respectively.

3.2. Cytotoxic activity

The cytotoxic effects of the isolated metabolites (**C1–C6**) and **H.S** extract on HepG2, KMST-6, and HaCaT cells were evaluated after 24-hours using the MTT test (Fig. 3). Among them, compound **1** demonstrated a significant decrease in HepG2, KMST-6 and HacaT cell viability. **C2** and **C6** yielded no significant (*P* > 0.05) reductions in HepG2, KMST-6 and HaCaT cell viability at the tested concentrations (12.5–200 μ g/ml) as compared to the control, while **C3** had moderate effects under the same conditions. A similar trend was also observed for the H.S extract in KMST-6 and HacaT cells. On the other hand, **C4** showed selective cytotoxic effects to KMST-6 cells. Furthermore, some of the tested metabolites were able to induce 50 % cell death (IC₅₀) at all the tested concentrations. It should be noted that among the tested compounds, **C1** showed the best cytotoxicity toward all the cell lines (HepG2, KMST-6 and HaCaT cells) at the tested concentrations (100–200 μ g/ml). Overall, all the tested compounds demonstrated significant cytotoxic effects toward one or the three cell lines except for **C2** and **C6** that showed no meaningful toxic effects.

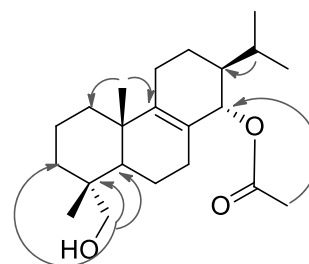


Fig. 2. Important HMBC correlations of compound 1 (**C1**).

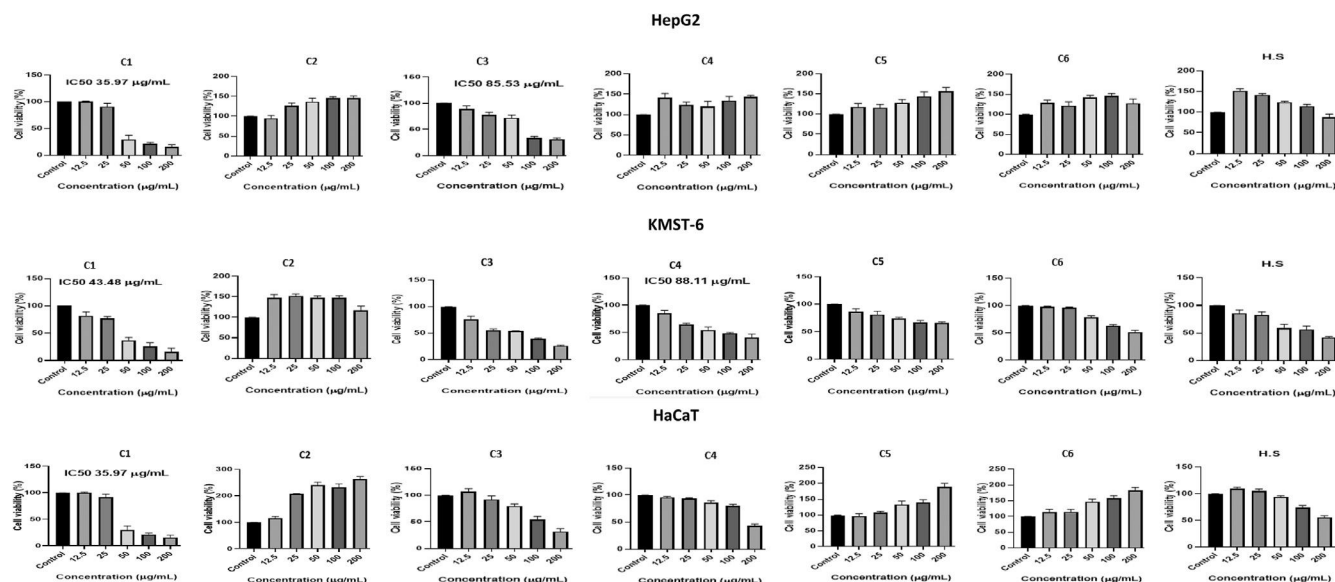


Fig. 3. MTT assay results for HepG2, KMST-6, and HaCaT cell viability after subjection to the metabolites (C1–C6) and H.S extract for 24 h.

4. Conclusions

The current study led to the isolation of six bioactive compounds from the acetone extract of the whole plant of *H. suaveolens*. The cytotoxic effects of these metabolites on HepG2, KMST-6, and HaCaT cells were evaluated using the MTT test. Among them, **C1** was isolated as a new metabolite and significantly reduced HepG2, KMST-6, and HaCaT cell viability with IC_{50} values of 35.97, 43.48, and 65.10 $\mu\text{g/ml}$, respectively. The study suggests **C1** (14-*O*-acetyl suaveolol) has promising anticancer properties, possibly due to the acetyl group on C-14, while **C2**, suaveolol, lacks this group, and did not yield any inhibitory activities. However, more data regarding its mechanism of action is recommended.

Declaration of competing interest

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

CRediT authorship contribution statement

Olatunji G. Azeez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Taofik A. Adedosu:** Supervision, Resources, Funding acquisition, Conceptualization. **Ahmed A. Hussein:** Writing – review & editing, Supervision, Resources. **Akeem Arinkoola:** Software. **Jelili A. Badmus:** Formal analysis. **Idowu J. Sagbo:** Software, Methodology, Investigation, Formal analysis, Data curation. **Keenau Pearce:** Methodology, Data curation. **Mongi Benjeddou:** Supervision, Resources. **Masande Yalo:** Formal analysis, Data curation. **Masixole Makhaba:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2025.04.010.

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