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The activities of suaveolol and other compounds from *Hyptis suaveolens* and *Momordica charantia* against the aetiological agents of African trypanosomiasis, leishmaniasis and malaria

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A B S T R A C T

African trypanosomiasis and malaria are among the most severe health challenges to humans and livestock in Africa and new drugs are needed. Leaves of *Hyptis suaveolens* Kuntze (Lamiaceae) and *Momordica charantia* L. (Cucurbitaceae) were extracted with hexane, ethyl acetate, and then methanol, and subjected to silica gel column chromatography. Structures of six isolated compounds were elucidated through NMR and HR-EIMS spectrometry. Callistrisic acid, dehydroabietinol, suaveolic acid, suaveolol, and a mixture of suaveolol and suaveolic acid (SSA) were obtained from *H. suaveolens*, while karavilagenin D and momordicin I acetate were obtained from *M. charantia*. The isolated biomolecules were tested against trypomastigotes of *Trypanosoma brucei brucei* and *T. congolense*, and against *Plasmodium falciparum*. The most promising EC_{50} values were obtained for the purified suaveolol fraction, at 2.71 ± 0.36 μg/mL, and SSA, exhibiting an EC_{50} of 1.56 ± 0.17 μg/mL against *T. b. brucei* trypomastigotes. Suaveolic acid had low activity against *T. b. brucei* but displayed moderate activity against *T. congolense* trypomastigotes at 11.1 ± 0.5 μg/mL. Suaveolol and SSA were also tested against *T. evansi*, *T. equiperdum*, *Leishmania major* and *L. mexicana* but the antileishmanial activity was low. Neither of the active compounds, nor the mixture of the two, displayed any cytotoxic effect on human foreskin fibroblast (HFF) cells at even the highest concentration tested, being 200 μg/mL. We conclude that suaveolol and its mixture possessed significant and selective trypanocidal activity.

1. Introduction

Protozoan parasites cause some of the most neglected diseases, especially in the tropics, including trypanosomiasis, leishmaniasis, and malaria. These are complex diseases, each associated with multiple parasite species, for which treatment is threatened by drug resistance (De Koning, 2017), for which no vaccine strategies are developed or, for malaria, fully evaluated and implemented (Björkman et al., 2023).

Trypanosomiasis is caused by protozoan parasites of the genus *Trypanosoma* that inhabit and replicate in the tissue of their human and animal hosts. It is transmitted by the bite of infected tsetse flies (*Glossina* species) in Africa (Barrett et al., 2003). Trypanosomes are heteroxenous parasites requiring a vector and a mammalian host including humans and animals such as buffaloes, cattle, cats, camels, dogs, donkeys, goats, horses, mules, pigs, and sheep – the sole exception being *T. equiperdum*, which is sexually transmitted between horses and other equids (Giordani et al., 2016).

Animal African trypanosomiasis (AAT) is caused mainly by *T. b.*

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brucei, *T. congolense*, *T. evansi*, *T. vivax* and *T. equiperdum* (Ungogo and De Koning, 2024). Of these, animal trypanosomiasis in the African tsetse belt is caused by *T. congolense*, *T. b. brucei* and *T. vivax*, while *T. evansi* is responsible for surra in some non-African regions, particularly in the Middle East and Asia, but also in parts of South America, where it can co-exist with *T. vivax* (Desquesnes et al., 2013). *T. evansi* and *T. vivax* can be transmitted by *Stomoxys* and tabanid flies and are thus not limited to tsetse fly habitat (Baldacchino et al., 2013; Desquesnes and Dia, 2003). *T. equiperdum* is responsible for dourine in Africa, the Middle East, Central and South America, while *T. vivax*, *T. brucei,* and *T. congolense* are the etiological agents of nagana in Africa. Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by *T. b. gambiense* and *T. b. rhodesiense* (WHO, 2021). Trypanosomiasis is one the diseases targeted for elimination as part of the World Health Organization's strategy for Neglected Tropical Diseases (NTD) 2021–2030 (WHO, 2023a). Mutations in some *Trypanosoma* sp. has led to the emergence of drug resistant strains (Kazibwe et al., 2009; Graf et al., 2013; Stewart et al., 2010; Munday et al., 2014). Moreover, treatment of the disease currently depends on the use of drugs with multiple side effects (De Koning, 2020) and the various drugs are mostly not effective against all the HAT or AAT species (Ungogo et al., 2022; Ungogo and De Koning, 2024). The reality today is that animal trypanosomiasis can no longer be effectively treated in much of Africa, affecting livelihoods, economies and food security (Giordani et al., 2016; Ungogo and De Koning, 2024).

Leishmaniasis is caused by an infection with one of approximately 20 human-infective protozoan parasites of the genus *Leishmania*; it is one of the WHO-designated NTDs and of significant public health concern in many countries of the tropics and subtropics, with up to 1 million new cases reported annually (Burza and Boelaert, 2018). The genus *Leishmania* is transmitted through the bite of an infected female of dozens of phlebotomine sandfly species (*Phlebotomus* and *Lutzomyia* spp. in the 'old world' and 'new world', respectively) (Maroli et al., 2013). Leishmaniasis can manifest itself as cutaneous, muco-cutaneous and visceral forms, mostly depending on the species (Burza and Boelaert, 2018). The treatment relies on a panel of drugs of various efficacies, availabilities and toxicities and resistance has been reported for all of them and even for combinations (García-Hernández et al., 2012). All or almost all *Leishmania* species causing human disease are believed to have animal reservoirs (Talmi-Frank et al., 2010; Babuadze et al., 2014; Montaner--Angoiti and Llobat, 2023).

Malaria results from persistent asexual intraerythrocytic developmental cycles by *Plasmodium falciparum* and other *Plasmodium* species in the host erythrocytes, after transmission by infected *Anopheles* mosquito. It can produce a range of clinical outcomes such as remaining asymptomatic despite infection, presenting with fever and other non-specific symptoms in uncomplicated cases, or exhibiting one or more signs of severe disease leading to cerebral malaria, severe anaemia, coma, pulmonary oedema, or metabolic acidosis (Mackintosh et al., 2004). Although malaria constitutes the largest disease burden of the tropical NTDs in terms of incidence and mortality, substantial progress in reducing its impact has been achieved over the first 15 years of the 21st century (Cibulskis et al., 2016). However, progress is being threatened by overdependence on artemisinin and its derivatives while resistance to these drugs is spreading (Hanboonkunupakarn and White, 2022).

The common challenges for these protozoan NTDs, of drug resistance and other drug insufficiencies, have necessitated the search for alternative medicines that could possibly come from natural products from traditional medicinal plants (Ungogo et al., 2020; Ebiloma et al., 2017, 2018) or bee propolis (Omar et al., 2016; Siheri et al., 2019; Ebiloma et al., 2020). According to estimates from the World Health Organization, nearly 4 billion people, or approximately 80% of the world's population, mostly residing in underdeveloped countries, rely on the use of herbal medicines as their primary healthcare (WHO, 2023b). Natural products are recognized to be effective drugs and they also continue to play a highly significant role in drug discovery as such drugs may be less likely to be toxic (Ekor, 2014). The use of natural products that exhibit significant antiprotozoal activities could be one potential source of much-needed new drugs against the various protozoan NTDs. However, it is essential that claims of antiparasite activity are meticulously investigated and, when appropriate, scientifically validated and this remains an important bottleneck. *H. suaveolens* Kuntze (Lamiaceae) is an aromatic annual shrub commonly regarded as a weed that has been reported to possess antimalarial activity (Ziegler et al., 2002) while *M. charantia* L. (Cucurbitaceae) is reported to have anthelminthic activity (Poolperm and Jiraungkoorskul, 2017). The antitrypanosomal, antileishmanial, and anti-plasmodial potential of compounds from *H. suaveolens* and *M. charantia*, currently used in Northern Nigeria for treating parasitic infections, is hereby reported.

2. Methodology

2.1. Plant collection

The plant materials were selected based on ethnobotanical reports from herbalists and published antiparasitic reports of *H. suaveolens* (Ziegler et al., 2002) and *M. charantia* (Santos et al., 2012). The plant leaves were collected from the Kaduna North Local Government Area of Kaduna State, Nigeria. Kaduna is found on coordinates 10°19′60″ N and 7°45′0″ E (Source: Geography Department, Nigerian Defence Academy Kaduna). Plants were identified at the Department of Biological Sciences, Nigerian Defence Academy, and voucher number NDA/-BIOH/2022/23 was assigned to *H. suaveolens* and NDA/BIOH/2022/26 to *M. charantia*.

2.2. Extraction procedure

The plant materials were air-dried at room temperature and ground into powder using a grinder. About 200 g each of the powders were successively extracted using a Soxhlet apparatus with *n*-hexane, ethyl acetate, and then methanol. The extracts were evaporated using a standard rotary evaporator at 40 °C and the solvent free extracts were stored at −20 °C.

2.3. Isolation of compounds

Column chromatography using silica gel, thin layer chromatography (TLC), and Gel filtration chromatography with Sephadex LH-20 (Sigma-Aldrich) were used to isolate the compounds from the plant extracts. The purity of the column fractions was evaluated using TLC and fractions that were observed to be mixtures were further purified using Sephadex.

2.3.1. Column chromatography

The extracts were subjected to column chromatography using silica gel (Merck, Germany) wet-packed in glass columns and eluted gradient-wise using mixtures of hexane, ethyl acetate and methanol. The extracts were pre-adsorbed on Celite® (Sigma-Aldrich) before loading onto the columns and eluted (collecting 20 mL fractions) with 500 mL each of increasing ratios of ethyl acetate in hexane: 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100, and thereafter with similar increasing amounts of methanol in ethyl acetate up to 30:70 totaling a collection of 120 fractions. The purity of the fractions was evaluated using TLC and mixtures were purified using gel filtration chromatography (GFC) with Sephadex LH-20 (Igoli et al., 2011).

2.3.2. TLC

TLC analyses were carried out on pre-coated silica gel aluminum plates. Developed TLC plates were examined under UV short ($\lambda = 254$ nm) and long ($\lambda = 366$ nm) wavelengths and thereafter sprayed with Anisaldehyde-H_2SO_4 reagent. Mixtures obtained from column fractions were combined and purified using GFC.

2.3.3. Gel filtration chromatography

The Sephadex LH-20 (Sigma-Aldrich, Germany) was pre-soaked for 24 h, packed, loaded with the fractions, and eluted with methanol, collecting 5 mL fractions. The structures of the purified compounds were determined using 1D and 2D (COSY, HSQC, HMBC, and NOESY) Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry.

2.3.4. Spectroscopic analysis

NMR experiments were run on a Bruker AV (400 MHz) or DRX-600 spectrophotometer. Samples were dissolved in 0.65 mL of deuterated chloroform ($CDCl_3$) or methanol (MeOD). Simpler compounds were identified by their one-dimensional ^1H and ^{13}C NMR spectra while 2D NMR was used to assign proton and carbon chemical shifts and also to determine the relative stereochemistry in the more complex compounds. HR-LC-MS was carried out using a JEOL 505HA mass spectrometer. Samples were dissolved in methanol (HPLC grade) to a concentration of 100 μg/mL. Using acetonitrile and water in a ratio of 90:10 at a flow rate of 200 μl/min, 10–20 μl samples were injected along with a direct infusion of 0.1% (v/v) formic acid.

2.4. Parasite culture

2.4.1. In vitro cultures of T. b. brucei, T. evansi and T. equiperdum trypomastigotes

Trypomastigotes of *T. b. brucei* Lister S427, *T. evansi* AnTat 3/3 and *T. equiperdum* BoTat1 (Stewart et al., 2010) were cultured in HMI-9 medium (Invitrogen, UK) supplemented with 14 μL/L β-mercaptoethanol, 3.0 g/L $NaHCO_3$ adjusted to pH 7.4, 10% (v/v) heat-inactivated fetal bovine serum (FBS) and cultured at 37 °C in a 5% CO_2 atmosphere (Ungogo et al., 2022).

2.4.2. In vitro cultures of T. congolense trypomastigotes

Trypanosoma congolense strains were cultured in TC-BSF-1 medium with 20% (v/v) goat serum at 34 °C in a 5% CO_2 atmosphere (Giordani et al., 2019). TC-BSF-1 medium consists of basal medium prepared with MEM medium (Sigma-Aldrich, United Kingdom), 26 mM $NaHCO_3$, 25 mM HEPES, 5.6 mM D-glucose, 1 mM sodium pyruvate, 100 μM hypoxanthine, 40 μM adenosine, 16.5 μM thymidine, 25 μM bathocuproine disulfonic acid disodium salt, and supplemented with 1.6 mM glutamine, 100 units/mL penicillin, 0.1 mg/mL streptomycin, β-mercaptoethanol (0.0014%, v/v), 20% (v/v) goat serum (Gibco, United Kingdom), and 5% (v/v) Serum Plus II (Sigma-Aldrich, United Kingdom).

2.4.3. In vitro cultures of L. major and L. mexicana

Leishmania major and *L. mexicana* promastigotes were cultured in HOMEM medium supplemented with 10% (v/v) FBS (FBS, Life Technologies) and 1% (v/v) penicillin/streptomycin solution (Gibco, United Kingdom) at 25 °C and 5% CO_2 and passaged every 72 h in HOMEM medium (Ebiloma et al., 2018).

2.4.4. Culture of P. falciparum

The *P. falciparum* strains 3D7 (chloroquine sensitive) and Dd2 (chloroquine resistant) were obtained from the Department of Epidemiology Laboratory, Noguchi Memorial Institute for Medical Research, University of Ghana. The culture method was adapted from Trager and Jensen (1976), using a medium consisting of RMPI 1640 supplemented with HEPES, AlbuMAX II, hypoxanthine, and sodium bicarbonate in an atmosphere of 90% N_2, 5% CO_2, and 5% O_2 at 37 °C. The parasites were cultured with oxygenated human erythrocytes until ≥5% ring stages were observed, after which they were repeatedly treated with 5% sorbitol for synchronization.

2.5. In vitro anti-trypanocidal activity of the purified compounds

The activity of the purified compounds was determined using a resazurin (Alamar blue)-based cell viability assay (Gould et al., 2008) as described previously (Alotaibi et al., 2021). Briefly, 200 μl of test compounds and control drugs in culture media were distributed in the first wells of the plate, and a double dilution was carried out over two rows in the appropriate medium for *T. b. brucei* or *T. congolense*, leaving the last rows as the drug-free negative control, amounting to 23 doubling dilutions. To each well, 2×10^5 trypomastigotes for *T. b. brucei* or 5×10^5 for *T. congolense* were added, followed by incubation of the plates at 37 °C/5% CO_2 (*T. b. brucei*) or 34.5 °C/5% CO_2 (*T. congolense*) for 48 h before the addition of 20 μl of a 0.49 mM solution of resazurin sodium salt (Sigma) in 1 × PBS, and a further incubation under the same conditions for 24 h. Fluorescence was measured using a FLUOstar Optima (BMG Labtech, Durham, USA) plate reader at excitation and emission wavelengths of 544 nm and 590 nm, respectively, and the EC_{50} of the compounds was then calculated using GraphPad Prism 8 by plotting to a 4-parameter equation for a sigmoid curve with variable slope. Internal control drugs for *T. b. brucei* (pentamidine isethionate (Sigma)) and *T. congolense* (diminazene aceturate (Sigma)) were included in each individual assay. Compounds were classified as having high activity when $EC_{50} < 10$ μg/mL, moderate activity when $10 < EC_{50} < 50$ μg/mL and poor activity when $EC_{50} > 50$ μg/mL.

2.6. Drug test with P. falciparum

100 μl of culture was added to an equal volume of the serially diluted test compounds in a 96 well culture plate (final concentration range of compounds in the wells was 1 mM–0.00195 mM). The culture was at a final hematocrit of 2% and 1% parasitemia. Each culture plate also contained artesunate as a standard antimalarial positive control drug. Wells made up of only parasites without drug/compound served as negative controls. The plates were arranged in a modular chamber kept at 37 °C and filled with a gas-mix of 90% N_2, 5% CO_2 and 5% O_2 and incubated for 72 h. Incubations were stopped by adding 100 μl of lysing buffer containing SYBR Green to each well and mixed well. The plates were incubated in the dark for 30–60 min and fluorescence was read with a FLUOstar OPTIMA (BMG Labtech) fluorometer plate reader version 2.20 at 470 nm and 520 nm. Artesunate was included in each assay as the internal control. EC_{50} values ($n = 3$) were calculated by plotting to a sigmoid curve with variable slope.

2.7. Drug tests against Leishmania major and L. Mexicana

Promastigote cultures of *L. major* Friedlin strain (Al-Salabi et al., 2003) and *L. mexicana* strain MNCY/BZ/62/M379 (Al-Salabi and De Koning, 2005) were cultured as described (Aldfer et al., 2022) in standard HOMEM (GIBCO, Life Technologies, Paisley, UK) with 10% FBS (PAA Laboratories, Linz, Austria) at 25 °C. Resazurin-based drug tests against both species were conducted exactly as described (Anyam et al., 2021), with the procedure similar to that used for *T. b. brucei*. Doubling dilutions were set up over 2 rows of a 96-well plate and 2×10^5 cells were added to each well, followed by incubation at 25 °C. After 72 h, 20 μL of a 125 μg/mL resazurin solution was added and the plates were incubated for a further 24 h. Fluorescence was read, and curves were plotted as for *T. b. brucei*. Pentamidine isethionate was used as the positive control.

2.8. Toxicity testing against human foreskin fibroblasts (HFF)

Assays to determine the toxicity of drugs to mammalian cells were carried out in human foreskin fibroblasts (HFF, ATCC code SCRC-1041), using a previously described method (Zheoat et al., 2021). Briefly, HFF cells were grown up to ~80% confluence in a medium made up of 500 mL Dulbecco's Modified Eagle's Medium (DMEM; Sigma), 50 mL new-born calf serum (NBCS; Gibco, Cleveland, TN, USA), 5 mL penicillin/streptomycin (Gibco), and 5 mL L-Glutamax (200 mM, Gibco), at 37 °C/5% CO_2 in vented flasks. 100 μL of a suspension of 3×10^5 cells/mL in this medium was added to each well of a 96-well plate. The

plate was incubated for 24 h at 37 °C/5% CO_2, allowing cell adhesion, and 100 μL of a separately prepared serial drug dilution was added. Phenylarsine oxide (PAO; Sigma) was used as the positive control. The cells were then incubated for a further 30 h before the addition of 10 μL of 125 mg/L resazurin solution and underwent a final incubation for 24 h. Fluorescence measurements and data analysis were performed, as described above. The selectivity index was calculated as EC_{50}(HF-F)/EC_{50} (parasite).

3. Results

The dried plant materials were sequentially extracted with hexane, ethyl acetate and methanol. The extracts obtained after removal of the solvents were fractionated using silica gel columns. Column chromatography of the ethyl acetate extract of *H. suaveolens* yielded callistrisic acid in fraction 14, suaveolic acid in fraction 33, suaveolol in fraction 47, and a mixture of suaveolic acid and suaveolol (SSA) in fraction 49, which was used without further attempts at purification or modification, while the methanol extract of *H. suaveolens* yielded dehydroabietinol in fraction 9. Similarly, column chromatography of *M. charantia* methanol extract yielded karavilagenin D in fraction 18 and momordicin I acetate in fractions 38–40.

3.1. Characterization of ESH 9 as dehydroabietinol (1)

Compound **1** was obtained as a white solid. Its HR-EIMS spectrum yielded a molecular ion $[M]^+$ at $m/z = 286.4510$ (calculated 286.2297, $C_{20}H_{30}O$) corresponding to the molecular formula $C_{20}H_{30}O$, with six degrees of unsaturation, in agreement with a tricyclic diterpene with an aromatic ring. This was confirmed by its ^1H NMR spectrum which showed signals for three aromatic protons at δ_H (ppm) 7.19 (d, $J = 8.2$ Hz, H-11), 7.00 (dd, $J = 8.2$ Hz, H-12) and 6.90 (d, $J = 2.0$ Hz, H-14) and a set of oxymethylene protons at δ_H 3.48 (H d, $J = 10.9$ Hz, H-18), 3.24 (d, $J = 10.9$ Hz, H-18). The spectrum also showed the presence of an isopropyl group due to a 6H (2 x CH_3) doublet at 1.24 (d, $J = 6.3$ Hz, H-16, H-17) coupled to a methine proton at δ_H 2.83 (d, $J = 6.2$, H-15). Two tertiary methyl protons were observed at 0.91 (s, H-19) and 1.25 (s, H-20). Its ^{13}C NMR spectrum showed signals for twenty carbon atoms including three aromatic quaternary carbons at δ_C (ppm) 134.9 (C-8), 147.5 (C-9), 145.7 (C-13), and three aromatic CH at 124.3 (C-11), 123.9 (C-12), 126.9 (C-14) indicating a trisubstituted ring. The structure was further deduced using correlations in its 2D NMR (COSY, HSQC, HMBC) spectra. In its COSY spectrum, ^1H–^1H correlations between the aromatic protons at 7.19 (H-11) and 7.00 (H-12) indicate they are ortho coupled. The HMBC spectrum showed long-range correlations between the aromatic proton at 7.00 (H-12) to the carbon at 126.9 (C-14) indicating they were three bonds apart. Other correlations from H-11 to C-8 and C-13 and from the methine septet at 2.83 (H-15) to the carbon at 126.9 (C-14) confirm the attachment of the isopropyl group to the aromatic ring. The HMBC correlations from the methyl protons at 0.91 (H-19) and the oxymethylene protons at 3.48 (H-18) to the carbon at 37.9 (C-4) indicate the methyl and oxymethylene groups are geminal at C-4. This is also confirmed by the long-range correlation from H-18 to C-19. Other long-range correlations from the methine proton at 1.67 (C-5) to the methyl carbon at 25.4 (C-20) confirm that the methyl group is attached at C-10. With its spectral data (Table 1) compared to literature reports of Chamy et al. (1987) and Pertino et al. (2017), compound **1** was identified as dehydroabietinol (Fig. 1).

3.2. Characterization of HSF 14 as callistrisic acid (2)

Compound **2** was also isolated as a white solid. Its HR-ESIMS spectrum gave a molecular ion at $[M]^+$ m/z 300.4350 (calcd. 300.2089, $C_{20}H_{28}O_2$) corresponding to the molecular formula, $C_{20}H_{28}O_2$. The ^1H and ^{13}C NMR data of compound **2** (Table 1) are similar to those of **1**, except for the appearance of a carboxylic acid carbonyl at δ_C (ppm) 184.5 (C-19). The long-range correlations from the tertiary methyl protons at 1.31 (H-18) and the methine proton at 2.28 (C-5) to the carboxylic acid carbon at 184.3 (C-19) confirm the attachment of the carboxylic acid group to be at C-4. With its spectral data compared to literature reports of Zhang and Sun (1989) and Zhang et al. (2013), compound **2** was identified as 4-epidehydroabietic acid or callistrisic acid (Fig. 1).

Table 1
^{13}C and ^1H chemical shifts for compounds 1 and 2.

Position	Compound 1		Compound 2	
	δ c	δ_H (multiplicity, J (Hz))	δ c	δ_H (multiplicity, J (Hz))
1	38.6	1.41, 2.30	38.6	1.41, 2.30
2	19.0	1.71, 1.79	19.0	1.71, 1.79
3.	35.2	1.48	35.2	1.48
4	37.9		37.9	
5.	44.1	1.67	44.1	1.67ff
6.	18.9	1.71, 1.81	18.9	1.71, 1.81
7.	30.1	2.90	30.1	2.90
8.	134.9		134.9	
9.	147.5		147.5	
10.	37.4		37.4	
11.	124.3	7.19	124.3	7.19
12.	123.9	7.00	123.9	7.00
13.	145.7	–	145.7	–
14.	126.9	6.90	126.9	6.90
15.	33.5	2.83	33.5	2.83
16.	23.6	1.24	23.6	1.24
17.	24.1	1.24	24.1	1.24
18.	72.3	3.24, 3.48	72.3	3.24, 3.48
19.	17.5	0.91	17.5	0.91
20.	25.4	1.25	25.4	1.25

3.3. Characterization of EHS 33 as suaveolic acid (3)

Compound **3** was also isolated as a white solid. The spectral data of compound **3** were similar to those of compounds **1** and **2** except for the absence of the aromatic protons and aromatic carbon signals. Its chemical shifts (Table 2) included a carboxylic acid carbonyl at δ_C 184.0 (C-18), two olefinic carbons at 130.7 (C-8), 142.9 (C-9), and an oxymethine carbon at 73.8 (C-14) and confirmed compound **3** to be suaveolic acid (Fig. 1) and this is supported by literature reports (Manchand et al., 1974).

3.4. Characterization of EHS 47 as suaveolol (4)

Compound **4** was similarly isolated as a white solid. The spectral data of compound **4** was also similar to that of **1** and **2** and analogue to compound **3**. In compound **4**, the carbonyl carbon was replaced by a hydroxyl bearing carbon at 71.3 (C-18). Its NMR chemical shifts (Table 2) were confirmed from literature reports (Manchand et al., 1974; Grassi et al., 2006), thus compound **4** was identified as suaveolol (Fig. 1).

3.5. Characterization of EHM 18 as karavilagenin D (5)

Compound **5** was isolated as a green solid and its HR-EIMS spectrum showed a molecular ion $[M]^+$ peak at m/z 470.6038 (calculated, 470.6038) corresponding to the formula $C_{30}H_{46}O_4$. The ^1H NMR signals were similar to those reported for karavilagenin D (Li et al., 2015; Chen et al., 2017). It showed resonances for five methyl singlets at 0.92 (6H (2 x CH_3, H-18, H-29), 1.18 (H-26), 0.84 (H-28), 1.25 (H-30), and a broad methyl singlet at 4.83 (H-27). The ^{13}C NMR showed signals for 30 carbon atoms including seven quaternary methyls, four olefinic carbons, two oxymethines and a lactone carbonyl. The HMBC correlations between the broad oxymethine proton at δ_H 3.45 (H-3) and the carbons at δ_C 18.4 (C-1) and 85.4 (C-5) indicates that the hydroxyl group is at C-3, while the long-range correlations between the olefinic proton at 5.70

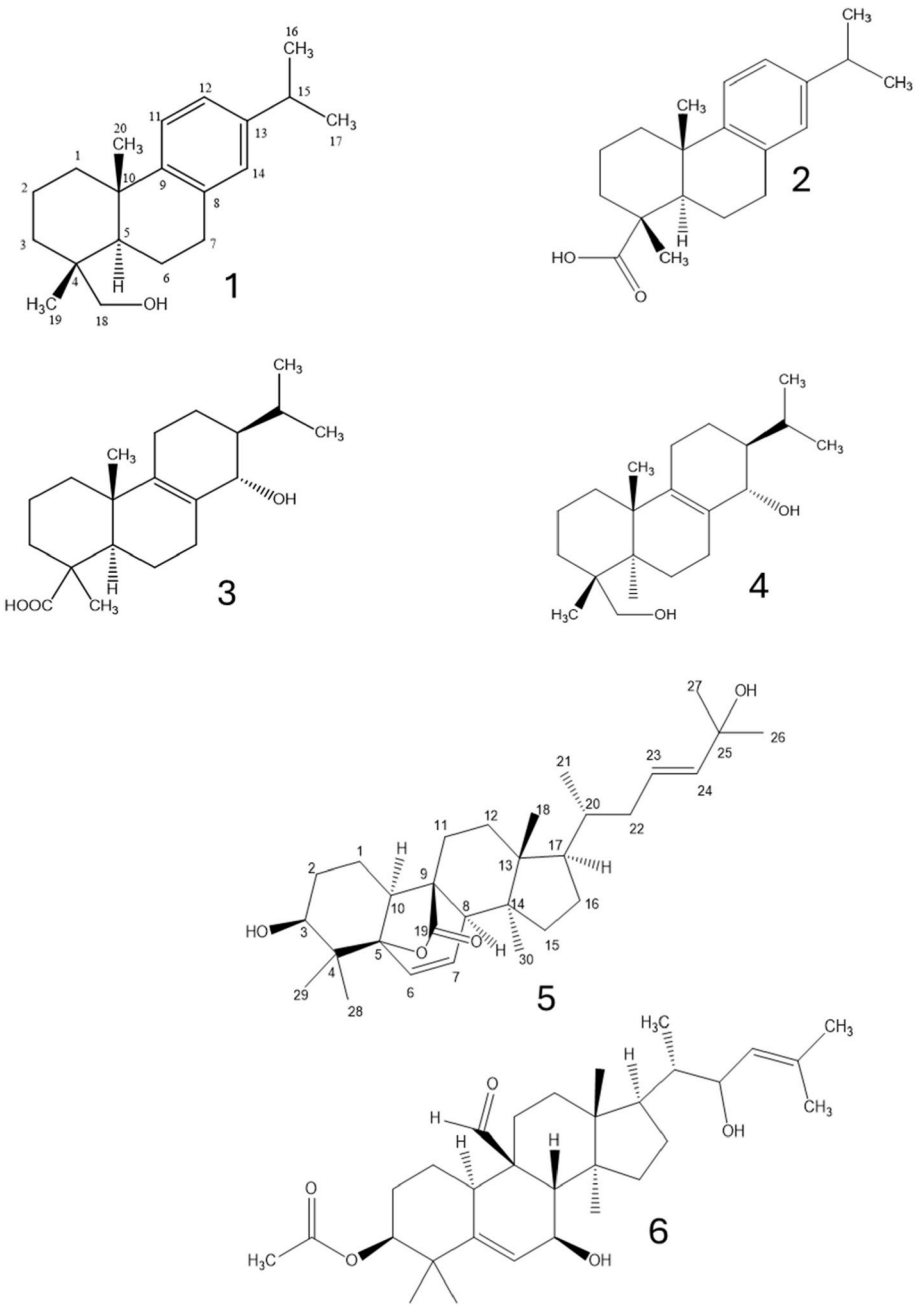

Fig. 1. Structures of the isolated compounds.
1, dehydroabietinol; **2**, callistrisic acid; **3**, suaveolic Acid; **4**, suaveolol; **5**, karavilagenin D; **6**, momordicin I Acetate.

(H-7) to the carbon at 84.5 (C-5) and 51.1 (C-9) confirmed that the lactone group is between C-5 and C-9 (Fig. 1). The ^{13}C signals at δc 36.5, 18.8, 39.9, 129.0, 134.3, 142.1, 18.8, 114.2 (C-20-27) were similar to those reported for the side chain of 5*β*,19-epoxy-23(*S*)-methoxycucurbita-6,24-dien-3*β*-ol (Nakamura et al., 2006; Chen et al., 2017). Due to the agreement between the experimental (Table 3) and literature NMR data, compound **5** was identified as karavilagenin D (Fig. 1).

3.6. Characterization of MCM 38–40 as momordicin I acetate (6)

The NMR spectra of compound **6** showed that it had a similar tetracyclic terpene carbon skeleton with compound **5.** In comparison of the ^{1}H and ^{13}C NMR spectra with literature (Yasuda et al., 1984; Li et al., 2015), the spectral data for compound **6** (Table 3) differed from that of momordicin I by the presence of an acetyl carbonyl at δ_C 170.6 ppm (in addition to the aldehyde carbon at δ_C 209.9 and a methoxy group at 21.2 ppm. The compound is momordicin I in salt form which has a similar tetracyclic terpene carbon skeleton to the karavilagenin compound. The acetylation of the hydroxyl group at position three is what caused the chemical shift to be at 3.63. Compound **6** was thus identified as momordicin I acetate (Fig. 1).

3.7. Characterization of EHS 49 as a mixture of suaveolol and suaveolic acid (SSA)

Characteristic signals due to the compounds were observed and were distinct enough for the compounds to be identified as a mixture with a ratio of 70% suaveolol and 30% suaveolic acid.

Table 2
^{13}C and ^{1}H chemical shifts for compounds 3 and 4.

Position	Compound 3		Compound 4	
	δ c	δ_H (multiplicity, *J* (Hz))	δ c	δ_H (multiplicity, *J* (Hz))
1	36.8	1.16, 1.78	35.7	1.10, 1.1174
2	19.3	1.58, 1.68	21.7	1.67, 1.07
3.	38.0	1.56, 1.82	34.7	1.46, 1.27
4	49.2	–	37.5	–
5.	47.5	2.04	44.4	–
6.	22.4	1.38, 1.59	18.2	1.09, 1.66
7.	29.2	1.86, 2.44	28.0	1.93, 2.47
8.	130.7	–	128.4	–
9.	142.9	–	144.0	–
10.	38.3	–	37.2	–
11.	25.3	1.89, 2.06	24.5	1.92, 2.04
12.	22.7	1.08, 1.69	18.3	1.65, 1.45
13.	49.4	1.31	48.8	1.26
14.	73.8	3.73	73.4	3.83
15.	28.0	2.04	27.0	
16.	17.2	0.81	17.2	0.83
17.	21.7	0.95	21.2	0.97
18.	184.0	–	71.5	1.09
19.	17.2	1.18	17.5	0.80
20.	19.6	0.98	19.5	1.02

Table 3
^{13}C and ^{1}H chemical shifts for compounds 5 and 6.

Position	Compound 5		Compound 6	
	δ c	δ_H (multiplicity, *J* (Hz))	δ c	δ_H (multiplicity, *J* (Hz))
1	18.4	1.26,1.62	24.1	
2	26.5	1.80	32.1	
3.	75.3	3.45	78.2	3.63
4	37.0	–	42.5	
5.	85.4	–	146.3	
6.	131.1	6.17	123.2	5.90
7.	133.4	5.70	67.4	4.06
8.	44.4	2.50	49.8	2.46
9.	51.1	–	53.2	
10.	39.9	2.62	38.2	2.60
11.	21.6	1.72, 2.20	24.9	
12.	29.8	1.52, 1.72	30.1	
13.	45.1	–	46.9	
14.	47.7	–	48.2	
15.	33.2	1.34	36.1	
16.	27.5	1.38, 1.96	29.4	
17.	50.5	1.48	52.1	
18.	14.6	0.92	15.6	0.18
19.	181.5	–	209.9	9.80
20.	36.5	1.52	32.9	1.97
21.	18.8	0.89	19.7	1.08
22.	39.9	1.78, 2.24	45.7	
23.	129.0	5.60	68.2	4.47
24.	134.3	6.10	135.4	5.18
25.	142.1	–	131.5	
26.	18.8	1.81	18.9	1.66
27.	114.2	4.83	26.7	1.72
28.	19.3	0.84	26.2	0.94
29.	23.5	0.92	28.4	1.09
30.	20.3	1.25	18.5	1.26

3.8. Effect of the isolated compounds on T. b. brucei and T. congolense

The *in vitro* activities of the isolated compounds were tested against bloodstream forms of *T. b. brucei* S427 and *T. congolense* IL3000 trypomastigotes using a resazurin-based assay. SSA, a mixture of suaveolol (70%) and suaveolic acid (30%), showed promising trypanocidal effects against *T. b. brucei* with an average EC_{50} value of 1.56 ± 0.17 μg/mL (n = 3) but displayed only moderate activity against *T. congolense* (EC_{50} > 10 μg/mL). Similarly, suaveolol exhibited potent activity against *T. b. brucei* with an EC_{50} value of 2.71 ± 0.36 μg/mL (8.84 μM) and only moderate activity against *T. congolense* (Fig. 2). Moderate-to-poor activities against both *Trypanosoma* species were observed for momordicin I acetate with EC_{50} values of 31.7 ± 4.3 μg/mL and 51.5 ± 4.3 μg/mL, respectively. The EC_{50} values obtained for dehydroabietinol, callistrisic acid, suaveolic acid, and karavilagenin D all showed quite low efficacy against *T. brucei*, although the activity of suaveolic acid was significantly more promising against *T. congolense*, with an EC_{50} of 11.1 ± 0.5 μg/mL ($P < 0.01$, unpaired *t*-test).

3.9. Effects of the isolated compounds against two strains of P. falciparum

The isolated compounds were next tested for antimalarial activity – specifically the chloroquine-sensitive strain 3D7 and chloroquine-resistant strain Dd2, allowing for the assessment of potential cross-resistance with the important quinoline class of antimalarials. There was no indication, however, of such cross-resistance, as EC_{50} values for the Dd2 strain were mostly lower than for 3D7 (1.5–4.1-fold, $P < 0.01$, Table 4) and none was significantly higher.

As for *T. b. brucei* (but not *T. congolense*), the highest activity was observed for the SSA mixture of suaveolol and suaveolic acid, with an EC_{50} value of just 2.1 μg/mL for the Dd2 strain and suaveolol was the most active component of that mixture (Fig. 3). Interestingly, momordicin 1 acetate and dehydroabietinol also displayed good antiplasmodial activity (Table 4), although they displayed very low activity against the trypanosome species.

3.10. Expanding the dataset to further Trypanozoon species

Because suaveolol and SSA were effective against *T. b. brucei* but not against *T. congolense*, the compounds would not be sufficiently effective against nagana in sub-Saharan Africa, where it is rarely known whether a given animal is infected with *T. brucei*, *T. congolense* or yet another trypanosome species. However, outside the tsetse belt of Africa and particularly in the Middle East and Asia, surra is almost universally ascribed to *T. evansi* or (in equids) *T. equiperdum* (Desquesnes et al., 2013) – and both are closely related to *T. brucei* spp (Trypanozoon subgenus) (Giordani et al., 2016). We thus retested these two products on bloodstream forms of *T. evansi* and *T. equiperdum*, using the exact same resazurin assay as for *T. b. brucei*. As expected, the EC_{50} values obtained for *T. evansi* and *T. equiperdum* with suaveolol and SSA far more closely resembled those of *T. b. brucei* than of *T. congolense* (Table 5). Indeed, suaveolol and SSA were significantly ($P = 0.013$ and 0.0054, respectively) more active against *T. evansi* than against *T. b. brucei*. For all three species against which suaveolol was active, the combination with suaveolic acid (SSA), was significantly more potent ($P < 0.05$ and $P < 0.01$).

3.11. Effects of suaveolol and SSA against Leishmania spp.

The same compound selection was also tested on promastigotes of *Leishmania major* and *L. mexicana* as representative old-world and new-world *Leishmania* species. The same resazurin-based protocol was used as for the trypanosome assays, adapted for promastigotes, all to allow meaningful side by side comparisons. However, suaveolol and SSA were both essentially inactive against either *Leishmania* spp (Table 5).

The conclusion of the anti-kinetoplast testing is that suaveolol was the most active compound isolated from these plants, and that its action was specific to the brucei group (Trypanozoon subgenus) of trypanosomes and that its activity was significantly potentiated by mixing with suaveolic acid.

3.12. Effects of suaveolol and SSA against HFF cells

Suaveolol and SSA were tested against cultures of human foreskin fibroblasts (HFF cells) as doubling dilutions from 200 μg/mL. The two highest concentrations of either preparation started to inhibit HFF growth or survival to some extent, resulting in a lowering of the

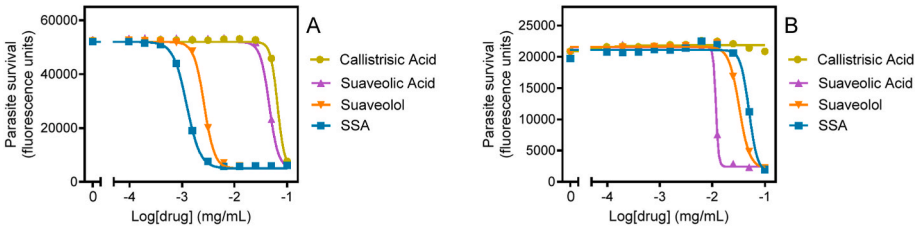

Fig. 2. Activity of some of the test compounds against bloodstream trypomastigotes of (A) T. *b. brucei* s427 and (B) *T. congolense* IL3000. The traces shown are of a single experiment and representative of 3 experiments conducted independently. Parasite survival is correlated to the level of fluorescence (Gould et al., 2008), which is expressed in artificial units. r^2 values were >0.98 in both frames.

Table 4
Antiparasite activities of isolated compounds EC_{50} values of purified compounds and the SSA mixture from *H. suaveolens* and *M. charantia* on *T. b. brucei* s427, *T. congolense* IL3000, *P. falciparum* 3D7 and *P. falciparum* Dd2. Values are of average ± standard error of the mean; n = 3.

Compound	*T. b. brucei* EC_{50}		*T. congolense* EC_{50}		*P. falciparum* 3D7 EC_{50}		*P. falciparum* Dd2 EC_{50}	
	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
Callistrisic acid	60.6 ± 1.4	201 ± 5	>100	>333	72.8 ± 2.4	243 ± 8	37.7 ± 0.8	126 ± 3[d]
Dehydroabietinol	55.7 ± 6.1	195 ± 21	>100	>349	28.2 ± 1.5	98.5 ± 5.1	16.9 ± 0.3	59.1 ± 1.2[c]
Karavilagenin D	>100	>212	>100	>212	28.6 ± 0.1	61.0 ± 0.2	29.6 ± 1.4	63.0 ± 2.2
Momordicin 1 Acetate	31.7 ± 4.3	61.6 ± 6.9	51.5 ± 4.3[a]	109 ± 9	12.2 ± 0.6	23.8 ± 1.2	7.9 ± 0.2	15.4 ± 1.0
Suaveolic acid	48.5 ± 7.3	152 ± 23	11.1 ± 0.5[b]	34.8 ± 1.7	28.4 ± 1.6	88.9 ± 4.9	19.2 ± 0.7	60.3 ± 2.2[c]
Suaveolol	2.71 ± 0.36	8.84 ± 1.18	30.3 ± 5.4[b]	99.0 ± 3.5	20.6 ± 0.5	67.2 ± 1.6	7.5 ± 0.3	24.3 ± 1.0[d]
SSA	1.56 ± 0.17	2.55 ± 0.41	41.3 ± 8.5[b]	70.9 ± 4.1	8.8 ± 0.6	14.4 ± 1.0	2.1 ± 0.1	3.5 ± 0.2[d]
Diminazene	–	0.12 ± 0.03	–	0.19 ± 0.03	–	–	–	–
Artesunate	–	–	–	–	0.00049 ± 0.00001	–	0.00019 ± 0.00001[e]	–

[a] $P < 0.05$
[b] $P < 0.01$ relative to *T. brucei* by unpaired two-tailed *t*-test.
[c] $P < 0.01$.
[d] $P < 0.001$.
[e] $P < 0.0001$ relative to *P. falciparum* 3D7.

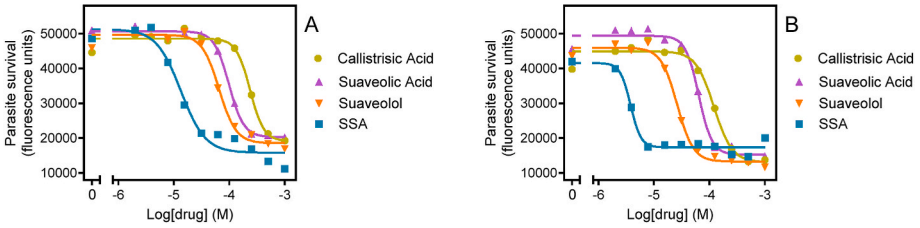

Fig. 3. Anti-plasmodial activity of four active compounds. A single representative experiment out of three identical repeats, with highly similar outputs, is shown. All r^2 values were >0.96 in both frames.

Table 5
Anti-kinetoplastid activities of suaveolol and SSA against *T. evansi*, *T. equiperdum, L. major* and *L. mexicana*. The assays used bloodstream trypomastigotes for *T. evansi* and *T. equiperdum*, and promastigotes for *Leishmania major* and *L. mexicana*. All data are average and SEM of at least three independent determinations.

Compound	*T. evansi* EC_{50}		*T. equiperdum* EC_{50}		*L. major* EC_{50}		*L. mexicana* EC_{50}	
	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
Suaveolol	1.14 ± 0.1[a]	3.72 ± 1.1	7.81 ± 1.1*	25.5 ± 3.1	91.2 ± 1.2	298 ± 4.2	100.7 ± 0.44[c]	328.5 ± 3.5
SSA	0.51 ± 0.1[b]	1.64 ± 1.2	0.96 ± 0.13	3.09 ± 2.3	49.9 ± 1.4	161 ± 4.1	48.9 ± 1.3	157.4 ± 2.4
Diminazene		0.016 ± 0.01		0.015 ± 0.004		n.d.		n.d.
Pentamidine		n.d.		n.d.		0.14 ± 0.002		0.23 ± 0.04

[a] $P < 0.05$
[b] $P < 0.01$ relative to *T. brucei* by unpaired two-tailed *t*-test.
[c] $P < 0.05$ relative to *L. major*. n. d., not determined.

fluorescence but did not reach 50% of inhibition of the PAO internal control and thus no reliable EC_{50} value could be obtained. The selectivity index (SI) for suaveolol and SSA against the various parasite species is itemized in Table 6. No attempt was made to obtain the HFF EC_{50} values for the other compounds described in this paper, as their low anti-parasite activity does not qualify them as potential lead compounds.

4. Discussion

There is an increasing awareness that plants that are traditionally applied for their antiparasitic effects in endemic countries, should be systematically investigated and the active principles identified (Ungogo et al., 2020). Here we report the isolation of six compounds from two

Table 6
Selectivity index for suaveolol and SSA against the various parasite species.

	T. b. brucei	T. congolense	*P. falciparum* 3D7	*P. falciparum* Dd2	T. evansi	T. equiperdum	L. major	L. mexicana
Suaveolol	>73.8	>6.6	>9.7	>8.2	>175	>25.6	>2.2	>2.0
SSA	>128	>4.8	>22.5	>57.1	>392	>208	>4.0	>4.1

SI = EC_{50}(HFF)/EC_{50}(parasite). The EC_{50}(HFF) was taken as 200 μg/mL for the calculation, being the highest concentration tested; since the growth inhibition was <50% at that concentration, the SI is listed as higher than (>) the ratio of 200 and the parasite EC_{50}.

plants, *Hyptis suaveolens* Kuntze and *Momordica charantia* L., with known antiparasite activity (Ziegler et al., 2002; Poolperm and Jiraungkoorskul, 2017). Dehydroabietinol was first isolated as a natural product from *Calceolaria ascendens* by Chamy et al. (1987) and isolated from *Hyptis suaveolens* by Ziegler et al. (2002). Dehydroabietinol acetate has been obtained from *Pinus silvestris* (Fujita, 1970) and its methyl ester form as 4-epi-dehydroabietic acid (callistrisic acid) from *Callistris columellaris* (Carman and Deeth, 1967). Callistrisic acid has been isolated from *Callitris* spp., *Dracocephaum taliense, Juniperus chinensis, Calceolaria* spp., *Rabdosia kunmingensis* and *Illicium majus* (Carman and Deeth, 1967; Zhang and Sun, 1989; Zhang et al., 2015; Yangan et al., 2017; Simoneit et al., 2018). Callistrisic acid which has not been isolated previously from *Hyptis suaveolens* though reported in GC-MS analysis by Machado et al. (2021) was isolated in this present study. Suaveolic acid has been isolated from the leaves of *Hyptis suaveolens* (Manchand et al., 1974; Panigrahi et al., 2002; Gavani and Paarakh, 2008) and suaveolol also from the leaves of *Hyptis suaveolens* (Manchand et al., 1974; Panigrahi et al., 2002; Gavani and Paarakh, 2008). Li et al. (2015) reported the isolation of karavilagenin D, momordicoside L, momordicin IV and momordicin I, the aglycone of momordicoside from the methanol extract of *Momordica charantia* leaves. Karavilagenin D has also been isolated from the fruit pulp of *Momordica charantia* by Chen et al., 2017.

Over the last few years, we have consistently demonstrated that diverse natural compounds from medicinal plants and bee propolis do not display reduced activity against *Trypanosoma* strains resistant to traditional drugs and display a high degree of selectivity over human cell lines (Ebiloma et al., 2017; Alotaibi et al., 2019, 2021; Omar et al., 2016, 2017; Nvau et al., 2020; Anyam et al., 2021) and, given the levels of drug resistance being reported, this is a major criterion for new treatments. The main phytochemicals isolated from *H. suaveolens* and *M. charantia* were terpenoids, specifically diterpenoids and triterpenes. Although terpenoids have diverse applications in foods, drugs, cosmetics, hormones, and vitamins, natural abietane diterpenes have shown promising antiparasitic activities (Breitmaier, 2006). Callistrisic acid was previously identified using GC-MS in a complex extract of *H. suaveolens* (Machado et al., 2021) but it was not isolated. It is reported to be found in the resins of several *Callitris* species (Cupressaceae) (Australian sandarac resin) (Simoneit et al., 2018). Suaveolol (13β-abiet-8-ene-14α, 18-diol) was effective against *T. b. brucei* and the closely related pathogens *T. evansi* and *T. equiperdum* but had tenfold lower effects against *T. congolense* and *P. falciparum* (Tables 4 and 5). The evolutionary distance between the first three trypanosome species, which belong to the sub-genus Trypanozoon, and *T. congolense* (Nanomonas sub-genus), is not insignificant. The specificity of suaveolol for Trypanozoon parasites was further highlighted by a lack of effect against representative old-world and new-world *Leishmania* species (*L. major* and *L. mexicana*) as well as against the human foreskin fibroblast (HFF) cell line. And although suaveolol has been reported to have some activity against the fungi *Candida albicans* and *Aspergillus niger* using an agar-based assay, it was completely inactive against the bacterial species *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa,* and the fungus *Trichophyton mentagrophytes* (Ragasa et al., 1997).

The poor activity displayed against *T. congolense* by the compound might be attributed to the difference in the biology of the two parasites which also affects the mechanisms of drug internalization and their mode of action, resulting in quite large differences in the pharmacology of even the established trypanocides (Carruthers et al., 2021; Ungogo et al., 2022) as well as newly designed nucleoside antimetabolites (Mabille et al., 2022; Ungogo et al., 2023); we also observed this previously for some natural compounds, such as bokkosin (Nvau et al., 2020; Anyam et al., 2021), although a series of European propolis extracts displayed similar activities against *T. brucei* and *T. congolense* bloodstream forms (Alotaibi et al., 2019). As to the moderate activity against *P. falciparum*, the biology of *Plasmodium* and *Trypanosoma* spp is very different, and that includes drug uptake and specific transporters. For instance, the diamidine pentamidine is taken up by a *T. brucei* aminopurine transporter (De Koning and Jarvis, 2001) and an aquaporin (Alghamdi et al., 2020), ultimately accumulating in the mitochondrion (Fidalgo and Gille, 2011), but *P. falciparum* utilizes an 'induced permeability pathway' in the plasma membrane of the infected erythrocyte followed by uptake into the parasite by a choline transporter (Biagini et al., 2004) and final accumulation in the food vacuole (Stead et al., 2001).

The structure of a lead compound can be optimized using structure-activity relationship (SAR) approaches, but this typically requires the synthesis of a substantial number of structural analogues and usually this is not an easy or cheap option for natural compounds, especially those with multiple chiral centers (suaveolol has five). However, *de novo* synthesis may not be necessary. For instance, according to Afolayan et al. (2018), hydroxylation of the sesquiterpenes at the C-2 position improves antileishmanial activity while hydroxylation at the C-3 position enhances the antitrypanosomal activity of labdane diterpenes. Similarly, Lategan et al. (2009) reported that the replacement of the –OH group in furanoterpenoid 9a-hydroxy-4aH-3,5,8a-trimethyl-4, 4a, 8a, 9-tetrahydronaphtho-([2,3 b]-dihydrofuran-2-one)-8-one with hydrogen to produce 4aH-3,5,8a-trimethyl-4, 4a, 8a, 9-tetrahydronaphtho-([2,3 b]-dihydrofuran-2-one)-8-one resulted in a three-fold increase in activity against the chloroquine resistant K1 strain of *Plasmodium falciparum in vitro*. Likewise we see here a range over one order of magnitude in the anti-plasmodial activity of the tested terpenoids. This shows that minor and easily accomplished chemical modifications of terpenoids can lead to a significant increase in anti-parasite activity – this is an option that to date remains highly under-researched.

The low trypanocidal activity of callistrisic acid, karavilagenin D, dehydroabietinol, and momordicin I acetate against both trypanosome species again suggests that relatively small modifications to the basic scaffold can make a large difference in the activity, and strengthens the case for a systematic investigation of the structure–activity relationship that could yield compounds that can substantially improve the anti-parasite efficacy of these compounds. Moreover, there could also be species differences and the compound series should be investigated against more protozoa, as dehydroabietinol was effective against leishmaniasis (Pertino et al., 2017) and *P. falciparum* (Ziegler et al., 2002).

The suaveolol/suaveolic acid mixture SSA showed more potent antitrypanosomal activity against *T. b. brucei* than either of the individual compounds, although the activity is certainly closer to that of suaveolol, which constitutes 70% of the mixture. Anyam et al. (2021) have reported similarly on the activity of 5S,7R,8R,9R,10S) -(−)-7,8-seco-7, 8-oxacassa-13,15-dien-7-ol-17-al and its mixtures containing various amounts of 19-dihydroxycassa-12-en-15-one (sandynone), (5S, 7R, 8R, 9R, 10S, 13Z, 17S)-7,8:7,17:16,17-triepoxy-7,8-seco-cassa-13-ene (niloticane B), (5S,7R,8R,9R, 10S) -(−)-7,8-seco-7, 8-oxacassa-13, 15-diene-7,17-diol, and (5S,7R,8R,9R, 10S) -(−)-7,8-seco-7, 8-oxacassa-13,15-dien-7-ol. It is no surprise that closely related natural

compounds might have a synergistic effect on a pathogen, as they are likely to target the same biochemical pathways and/or uptake mechanisms (Lüscher et al., 2007).

Suaveolic acid was the only isolated compound that showed moderate activity against *T. congolense* but it had low activity against *T. b brucei*. The moderate activity shown might be attributed to the presence of the acidic group in the compound, which suaveolol lacks. Abietane diterpenoids (suaveolol and suaveolic acid) are a large group of secondary metabolites that have been reported to exhibit diverse biological activities (Gonzalez, 2015). Saad et al. (2020) reported that anti-trypanosomal effects of the hydrogenated diterpene phytol are the result of inhibition of *T. congolense* sialidase and could reduce the parasites' harmful effects on the host. It may be that the stronger activity of suaveolic acid against *T. congolense*, relative to the corresponding alcohol, arises from improved inhibition of *T. congolense* sialidase. Further studies would be needed to explore this possibility; however, the lack of activity against *T. brucei* probably does not warrant extensive efforts in that direction, as there is no call for a *T. congolense*-specific trypanocide. The antimalarial activity of the SSA mixture, and any chemical derivatives that can be made should be further explored, given the activity of just 2.1 μg/mL against a highly chloroquine-resistant strain.

Suaveolol and SSA, which showed promising activities against *T. b. brucei*, were also active against the dyskinetoplastic *Trypanosoma* species *T. evansi* and *T. equiperdum*, which are the etiological agents of the veterinary conditions surra and dourine, respectively. Unlike the animal trypanosomiasis in sub-Saharan Africa, 'nagana', surra and dourine are geographically impossible to be caused by *T. congolense*, which is restricted to the tsetse habitat in sub-Saharan Africa, but in the Middle East and Asia only by the Trypanozoon subgenus parasites that are much more closely related to *T. brucei* (Giordani et al., 2016). Thus, while suaveolol does not look like a practical solution for nagana it does seem to hold promise for surra and perhaps dourine.

5. Conclusion

Callistrisic acid, suaveolol and its mixture SSA were obtained from *H. suaveolens* leaf extract. They exhibited promising antitrypanosomal activity against Trypanozoon group trypanosomes. The SSA mixture of suaveolol and suaveolic acid, and momordicin I acetate showed promise as anti-plasmodial agents. It might be possible to further increase the potency by optimizing the combination with suaveolic acid and/or minor modifications to the structure that can be implemented without complete *de novo* synthesis of the molecule.

CRediT authorship contribution statement

Enimie E. Oaikhena: Writing – original draft, Investigation, Funding acquisition. **Umar A. Yahaya:** Supervision, Formal analysis. **Sani M. Abdulsalami:** Supervision, Funding acquisition, Conceptualization. **Nkechi L. Egbe:** Supervision, Methodology. **Modupe M. Adeyemi:** Supervision, Project administration, Data curation. **Marzuq A. Ungogo:** Methodology, Investigation. **Godwin U. Ebiloma:** Investigation. **Felix K. Zoiku:** Investigation. **Prince A. Fordjour:** Investigation. **Hamza A.A. Elati:** Investigation. **Neils B. Quashie:** Supervision, Resources. **John O. Igoli:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation, Conceptualization. **Alexander I. Gray:** Formal analysis. **Christopher Lawson:** Investigation. **Valerie A. Ferro:** Supervision, Methodology. **Harry P. de Koning:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis.

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.

Data availability

Data will be made available on request.

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