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Anti-Trypanosomal and Antileishmanial Effect of Phytosterols Isolated from *Hyptis Suaveolens*

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Abstract

African trypanosomiasis and leishmaniasis are diseases of significant public and veterinary importance globally. Treatment of both infections has been dependent on the use of synthetic drugs which had several drawbacks creating the need for drugs from non-synthetic sources such as plants. To evaluate the effect of phytosterols which are a group of sterols naturally found in plants and are structurally related to cholesterol, soxhlet extraction method was used to isolate the phytochemicals using hexane, ethyl acetate, and methanol as solvent from *Hyptis suaveolens*. Using different concentrations (100 µg/mL to 0.049 µg/mL) of the phytosterols, in vitro antileishmanial and antitrypanosomal activities were carried out against trypomastigotes stage of *Trypanosoma brucei brucei* s427 and promastigotes of *Leishmania major* Friedlin strain in 96 well plates. Phytosterols isolated from this research were α Amyrin, β Amyrin, α and β Amyrin, Pheophytin A and Pheophytin B. Pheophytin A and α and β Amyrin in a ratio of 50:50 displayed moderate activities with EC_{50} values of 25.73 µg/mL and 37.65 µg/mL respectively for *T. b. brucei* s427 while EC_{50} values of 25.95 µg/mL and 39.94 µg/mL respectively for *L. major*. Poor activities (EC_{50} ranging between 49-221 µg/mL) were exhibited by α Amyrin, β Amyrin and pheophytin B when tested against both parasites. In conclusion, the study provides insights into phytosterols' global role against African trypanosomiasis and leishmaniasis.

Keywords: African Trypanosomiasis, Phytosterols, Antitrypanosomal, *In vitro* assay, Leishmaniasis

1.0 Introduction

Diseases such as leishmaniasis, dengue fever, lymphatic filariasis, and trachoma are considered examples of neglected tropical diseases (NTDs), which are referred to since they primarily affect the underprivileged and historically have not drawn as much awareness as other ailments (Oghifo, 2018). These communities are typically found in distant and rural regions, such as slums, conflict zones, or informal settlements, where people lack access to proper hygienic conditions, clean water, and healthcare.

Trypanosoma, a single-celled, flagellated parasitic protozoan from the trypanosomatidae family, causes African trypanosomes, also known as Old World trypanosomes, the cause of sleeping sickness. The protozoan parasite is spread by a tsetse fly of the *Glossina* sp, which affects 37 African countries (Denbarga *et al*., 2012). Trypanosomiasis poses a major public health threat in sub-Saharan Africa, with an estimated 70 million people at risk of infection (Orish, 2024). African trypanosomiasis poses a significant health

challenge to humans and livestock inAfrica (WHO, 2012). Animal African trypanosomiasis (AAT) and human African trypanosomiasis (HAT) are two subtypes of African trypanosomiasis. The term trypanosome refers to the genus of protozoan parasites responsible for trypanosomiasis, while trypanosomiasis describes the diseases caused by these parasites (Stijlemans et al., 2024).

Despite extensive research on new antitrypanasomal drugs, first-line treatment remains dependent on pentavalent antimonials, a class of drugs developed over 50 years ago. These drugs exhibit toxicity and are susceptible to resistance (De Rycker, 2023). They are also associated with severe side effects and the growing concern of drug resistance. This situation reinforces the urgent need for new trypanosomiasis drugs that are effective, affordable, and safe for the treatment of trypanosomiasis.

Leishmaniasis continues to inflict high mortality and morbidity in developing countries. Several *Leishmania* species are identified as the causative agents of leishmaniasis. This disease ranks among the leading endemic parasitic infections globally, with a presence in approximately 99 countries, particularly in developing countries (Almeida-Souza, 2024). An estimated 1.7 billion people worldwide are at risk of contracting the infection (Hudu *et al.,* 2024). The lack of an effective vaccine and readily affordable treatment options pose significant challenges. Existing anti-leishmanial drugs present additional hurdles due to drawbacks such as toxicity, high cost, and the emergence of drug resistance. First-line pentavalent antimonials, for example, can induce severe toxic side effects, including cardiotoxicity, pancreatitis, hepatotoxicity, and nephrotoxicity (Berhe, 2024). Second-line drugs like pentamidine and miltefosine, when administered in high doses, carry the risk of inducing diabetes (Gopu et al., 2023). Furthermore, the recent development of resistance against certain antileishmanial drugs has led to treatment failures, highlighting the urgent need for novel therapeutic approaches (Berhe, 2024).

Natural products, particularly plant extracts and their derived compounds are a promising source of new drugs against leishmaniasis and trypanosomiasis, driving research into novel therapies (Adegboye et al., 2021; Ungogo et al., 2020). *Hyptis suaveolens,* a member of the Lamiaceae family, is a medicinal plant with a rich history of ethnobotanical significance (Srivastava et al., 2022). Though commonly considered a weed in tropical and subtropical regions, various parts of *H. suaveolens* are used in traditional medicine for various ailments (Li et al., 2020). In West Africa, particularly northern Nigeria, *Hyptis suaveolens* (also known as curry leaf) finds application as a local seasoning and in traditional remedies for a variety of ailments including diabetes mellitus, fever, eczema, flatulence, cancers, and headaches (Okoye et al., 2014). Both leaves and twigs are reported to possess antisplasmodic properties and serve as sources of anti-inflammatory, antioxidant, and antifertility agents (Ghaffari et al., 2014). Additionally, *H. suaveolens* exhibits antiseptic properties and is used topically for burns, wounds, and various skin conditions (Srivastava et al., 2022). Chemically, *H. suaveolens* is rich in various constituents with potential medicinal properties, such as carbohydrates, tannins, phenols, saponins, steroids, alkaloids, and glycosides (Ortiz-Mendoza et al., 2023). Based on traditional knowledge and recent pharmacological studies, this research aims to determine the antileishmanial and antitrypanosomal effects of phytosterols isolated from *Hyptis suaveolens*.

2.0 Materials and Methods

2.1 Collection and Identification of *Hyptis suaveolens*

Hyptis suaveolens was selected based on its potential for treating parasitic infections and information from herbalists. The plant was collected from Kaduna North local government area in Kaduna, Kaduna State, and identified by a botanist at the Department of Biological Sciences, Nigerian Defence Academy. The voucher number was assigned to the plant as NDA/BIOH/2022/23.

2.2 Isolation and Structural Elucidation of Biomolecules from *Hyptis Suaveolens*

2.2.1 Extraction Procedure

Leaves of *Hyptis suaveolens* were air-dried and processed into powder with a grinder.
The extraction was carried out with the Soxhlet equipment using twenty grams (20g) of powdered

dry leaves. Extraction was done using n-hexane, ethyl acetate, and methanol as solvents. Each of the extracts obtained were evaporated at 40^{o}C in a fume hood. Each extract's solvent and volume of 100-200ml were recovered under a vacuum using a rotating evaporator linked to a condenser. Residual solvents were allowed to evaporate under the fume hood. Prior to analysis, samples were tagged and stored at -20^{0}C (Ebiloma et al,. 2017).

2.2.2 Isolation of compounds

Column chromatography using silica gel MN-60 and thin layer chromatography using Sephadex LH-20 were used to isolate compounds from plant crude extracts.

2.2.2.1 Column Chromatography

Column chromatography (CC) was performed on wet-packed silica gel 60 (Merck, Germany) on glass columns using n-hexane, hexane-ethyl acetate, ethyl acetate, and ethyl acetate-methanol solvents to elute the sample gradient-wise fashion. The adsorbed plant extract in celite (Sigma-Aldrich, Germany) was loaded onto the column and eluted into a 20 mL vial using 500 mL each of increasing ratios of ethyl acetate in hexane or ethyl acetate: 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. (Igoli et al., 2011).

2.2.2.2 Thin Layer Chromatography

Thin Layer chromatography (TLC) analysis was done on pre-coated silica gel aluminum plates. Spots on the TLC were visualized using Anisaldehyde-H2SO4 or Dragendorff's reagents for alkaloids, saponin, steroids, or terpenes. Observation of developed TLC plates were done under UV lamp using short (λ=254nm) and long (λ=366nm) wavelengths (Igoli et al., 2011).

2.2.3 Structural Elucidation of Compounds

Spectroscopic methods (1D/2D NMR and mass spectrometry) were used to elucidate the structures of isolated compounds

2.2.3.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) experiments were run on Bruker AV 300 (400MHz) or DRX-600 spectrophotometer. Samples were dissolved in 0.6 ml of NMR-grade deuterated chloroform or methanol in NMR tubes. Pure compounds were identified by one-dimensional ^{1}H and ^{13}C NMR spectroscopy and 2D NMR spectroscopy. Following a comparison with existing spectral data, compounds spectra were identified (Igoli et al., 2011).

2.2.3.2 Liquid Chromatography Mass Spectrometry (LC-MS)

Identification of the mass and type of chemicals present in samples was done by Mass spectrometry using mass-to-charge ratio measurement and abundance of gas-phase ions. On a JEOL 505HA spectrometer, the impact mass spectra of high/low-resolution electrons employing direct probe at higher temperature (110–160 °C) at 70 eV were obtained. The modes of positive and negative ions on a Thermo Finnigan LCQ-Deca Orbitrap or Iontrap HRESI mass spectrometer (mass analyzer set up at 100,000 ppm, externally calibrated at 3 ppm) or electrospray ionization (ESI) studies were carried out (Igoli et al., 2011).

2.3. Drug Sensitivity Against Anti-Kinetoplastid Activity

2.3.1 Parasite strains and cultures

Trypomastigotes wild type of *T. b. brucei* strain s427 was cultured in HMI-9 medium supplemented with 10% Fetal Bovine Serum at 37 °C. *Leishmania major and Leishmania 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^{o}C and 5% CO_2 and passaged every 72 hrs in HOMEM medium (Ebiloma et al., 2018).

2.3.1.1 *In Vitro* Anti-Trypanosomal Activity of the Extracted Compounds

Isolated compounds were investigated for their anti-trypanosomal effects on the trypomastigotes stage of *Trypanosoma brucei brucei* s427. Alamar blue ™ 96 well microplate test was used to assess the activities of the compounds. Test compounds (2x the maximum concentration) were serially diluted 1:1 (100 g/ml as the top concentration to 0.0975 g/ml) in HMI-9 medium (adjusted to pH 7.4 is14 g/L β mercaptoethanol, and 3.0 g/L $NaHCO_3$). Using a multichannel pipette, the samples (100 L) were then added to the cultivated cells and left in

the incubator at 37ºC and 5% CO_2 for 48 hours). The plate was incubated for 48 hours before 20 µL of 5 mM resazurin sodium salt (Sigma Aldrich, UK) was added and then incubated for another 24 hours..The fluorescence intensity (λex/em: 544 and 590 nm) of the plant extracts was determined using a FLUOstar OPTIMA (Ebiloma et al., 2018).

2.3.1.2 *In Vitro* Anti-leishmanial Activity of the Extracted Compounds

Drug susceptibilities of the isolated compounds were determined for bloodstream forms of *L. major* and *L. Mexicana* wild types using the Alamar blue (resazurin) assay as described previously, using 23 doubling dilutions from an initial test compound concentration of 20 µM, and 10^6 *Leishmania*/well; plates were incubated at 25 °C/5% CO_2 for 78 h and 48 h before and after the addition of Alamar blue, respectively. At excitation and emission wavelengths of 544 nm and 590 nm, respectively, fluorescence was measured using a FLUOstar Optima (BMG Labtech, Durham, NC, USA) plate reader. The calculation for the EC_{50} of the compounds were done using GraphPad Prism 5 (Ebiloma et al., 2017).

2.4 Statistical Analysis

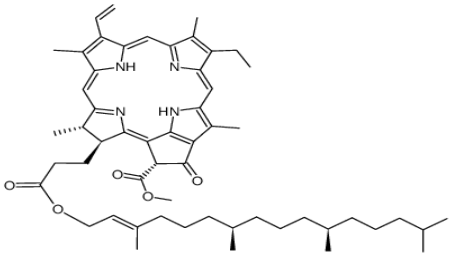
The results obtained were analyzed using one-way Analysis of variance (ANOVA) between control and different concentrations for the effect of the isolated compounds on Parasites. The package adopted was Statistical Package for Social Sciences (SPSS) version 23 (New York, USA).

3.0 Results

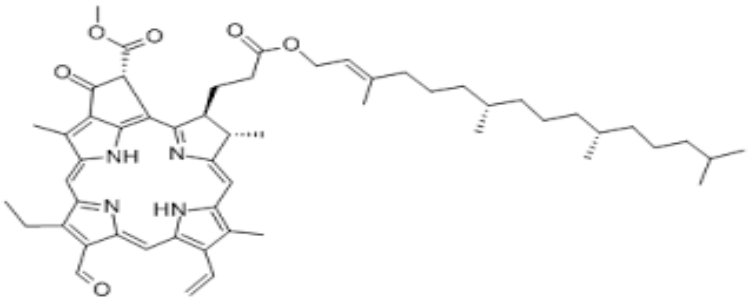
3.1 Isolation and Structural Elucidation of Biomolecules from *Hyptis suaveolens*

To evaluate the effect of phytosterols which are a group of sterols naturally found in plants and are structurally related to cholesterol, soxhlet extraction method was used to isolate the phytochemicals using n-hexane, ethyl acetate and methanol as solvent from *Hyptis suaveolens*. Using different concentrations (100 µg/mL to 0.049 µg/mL) of the phytosterols, in vitro antileishmanial and antitrypanosomal activities were carried out against trypomastigotes stage of *Trypanosoma brucei brucei S427* and promastigotes of *Leishmania major* Friedelin strain in 96 well plates. Phytosterols isolated from this research were α Amyrin, β Amyrin, α & β Amyrin, Pheophytin A and Pheophytin B. α & β Amyrin synergetic compound was isolated in a ratio of 50:50.

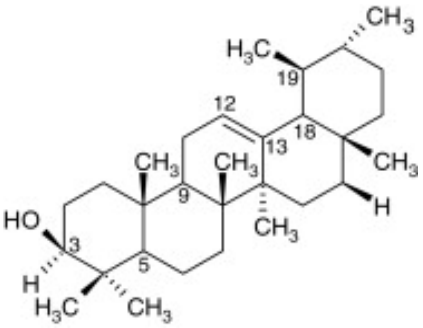
Literature denotes a collection of writings chiefly appraised to be in art form, expressly prose fiction, drama, and or poetry that explores human experiences in society and serves as catalyst for personality and identity (Amuta 1989). With the passage of time, the definition integrates oral literature or orature. Literature is a cultural means of recording, preserving, and transmitting knowledge and of entertainment. It also has a psychosocial, spiritual, and political significance (Finnegan 1976).



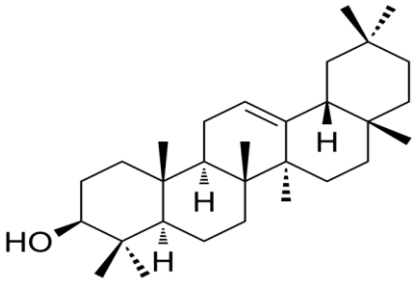
a) Pheophytin A



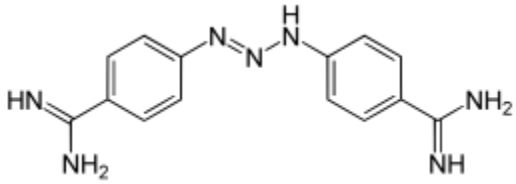
b) Pheophytin B



c) α Amyrin



d) β Amyrin



e) Diminazene

Figure 1: Phytosterols Extracted from *Hyptis suaveolens* and structure of Diminazene (Positive control)

3.2 *In Vitro* Antitrypanosomal and Anti-leishmanial Activities of the Extracted Compounds

3.2.1 Antitrypanosomal Effect of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *Trypansoma brucei brucei*

Table 1 shows the antitrypanosomal effects of 5 compounds: α amyrin, α & β amyrin, β amyrin, Pheophytin A and Pheophytin B isolated from *Hyptis suaveolens* against *T. b. bruce*. Five (5) compounds. The table shows that Phenophytin A and α & β Amyrin exhibited moderate activities against the *T. b. brucei*. α Amyrin, β Amyrin, and Pheophytin B displayed poor activities which range from 54.13 to 95.65 µg/mL on the parasite when compared to Diminazene the standard drug that was highly effective with a much lower concentration (0.012 µM) compared to the other compounds. The compounds were all significantly different from the control drug except α & β Amyrin which was slightly significantly different from diminazene than the other isolated compounds.

Table 1: Antitrypanosomal Effects of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *Trypansoma brucei brucei*

Compound	Repeat 1	Repeat 2	Repeat 3	Average (EC_{50})	SEM	Unit
α amyrin	59.19	48.7	54.515	54.13[d]	3.03	µg/mL
α & β amyrin	24.84	27.24	25.13	25.75[b]	0.75	µg/mL
β amyrin	59.26	58.92	64.23	60.80[d]	1.72	µg/mL
Pheophytin A	37.68	35.37	39.9	37.65[c]	1.31	µg/mL
Pheophytin B	92.03	110.47	84.47	95.66[e]	7.72	µg/mL
Diminazene	0.01512	0.008665	0.01172	0.012[a]	0.002	µM

Key:
EC_{50}: Half maximal effective Concentration
SEM: Standard Error of Mean

3.2.2 Antileishmanial Effects of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *Leishmaniasis major*

Table 2 shows antileishmanial properties of various phytosterols isolated from *Hyptis suaveolens* against *Leishmania major* in vitro. The result revealed Phenophytin A and α & β amyrin showed moderate activities against the *L. major*. Other isolated compounds, including α amyrin and β amyrin, also displayed a dose-dependent inhibition of *Leishmania major* parasites (49.30 to 58.55 µg/mL), although to a lesser extent compared to pheophytin A and α & β Amyrin. The findings revealed pheophytin B emerging as the lowest inhibitor, exhibiting 221.67 µg/mL average inhibition. These results suggest a potential role for these phytosterols in combating *Leishmaniasis major*. The result suggests that α & β Amyrin was slightly different from pentaamine the control drugs compared to the other isolated compounds.

Table 2: Antileishmanial effects of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *Leishmaniasis major*

Compound	Repeat 1	Repeat 2	Repeat 3	Average (EC_{50})	SEM	Unit
α amyrin	46.15	53.18	48.57	49.30[cd]	2.06	µg/mL
α & β amyrin	28.94	24.5	24.41	25.95[b]	1.50	µg/mL
β amyrin	54.9	64.97	55.79	58.55[d]	3.22	µg/mL
Pheophytin A	42.14	34.77	42.9	39.94[c]	2.59	µg/mL
Pheophytin B	163.8	202.8	298.8	221.67[e]	40.11	µg/mL
Pentamidine	0.9135	0.5796	0.9054	0.638[a]	0.179	µM

Key:
EC_{50}: Half maximal effective Concentration
SEM: Standard Error of Mean

3.2.3 Antileishmanial effects of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *Leishmania mexicana*

The antileishmanial effects of five isolated phytosterol compounds (alpha amyrin, alpha & beta amyrin, beta amyrin, pheophytin A, and pheophytin B) from *Hyptis suaveolens* were investigated against *L. mexicana* parasites. The results revealed moderate antileishmanial activity with α & β Amyrin. α amyrin, β amyrin, pheophytin A, and pheophytin B demonstrated poor inhibition activities across all tested

concentrations with EC_{50} values of 54.79 to 98.83 µg/mL. α & β Amyrin was slightly different, α amyrin, β amyrin, and pheophytin A were moderately different while pheophytin B was highly significantly different from pentamidine the control.

Table 3: Antileishmanial effects of Phytosterol Compounds Isolated from *Hyptis suaveolens* against *L. mexicana*

Compound	Repeat 1	Repeat 2	Repeat 3	Average (EC_{50})	SEM	Unit
α amyrin	56.48	59.19	48.7	54.79 [c]	3.14	µg/mL
α & β amyrin	24.84	27.24	25.13	25.74 [b]	0.76	µg/mL
β amyrin	59.26	58.92	64.23	60.80 [c]	1.72	µg/mL
Pheophytin A	67.68	100	39.9	69.19 [c]	17.34	µg/mL
Pheophytin B	92.03	120	84.47	98.83 [d]	10.81	µg/mL
Pentamidine	0.01512	0.008665	0.01172	0.012 [a]	0.002	µM

Key:
EC_{50}: Half maximal effective Concentration
SEM: Standard Error of Mean

Discussion

Medicinal plants are a rich source of structurally diverse compounds with various biological activities (Mohamed et al., 2023). These activities, including antibacterial, antioxidant, antitumor, anti-inflammatory, antitrypanosomal, and antileishmanial properties, are often attributed to secondary metabolites (Nascimento et al., 2023). Phytosterols which exist in all plant cell membranes are especially enriched in vegetable oils and fats, cereals, and cereal products, vegetables, fruits, and berries (Pironen and Lampi, 2004).

Phytosterols isolated from this research are α Amyrin, β Amyrin, α and β Amyrin, Pheophytin A and Pheophytin B. Similarly, α Amyrin has been previously isolated from *Celastrus hindsii* (Viet et al., 2021) and *Brachstelma togoense* (Ekalu et al., 2019). β Amyrin isolated from *Celastrus hindsii* (Viet et al., 2021) and *Alstonia beeonei* (Okoye et al., 2014). Pheophytin A isolated from *Brachstelma togoense* (Ekalu et al., 2019) and *Camellia sinensis* (Lia et al., 2015). Pheophytin B from *Camellia sinensis* (Lia et al., 2015) and *Prosopis julifora* (Singh, 2012). Alpha and beta amyrins are categorized as 4,4- dimethylsterol, a phytosterol class. Pheophytin occurs naturally in the plant leaves and is important as the first electron carrier intermediate in plants' electron transfer pathway of photosystem II.

Phytosterol compounds' effect against *Trypansoma brucei brucei* suggested that the compounds could contain trypanocidal constituents that are active in the *in vitro* environments. However, Pheophytin A and α & β amyrin displayed moderate *in vitro* trypanocidal activities. Pardo-Rodriguez (2023) research on α amyrin also displayed moderate activity against *T. cruzi*. Dofuor (2022) found that *Anthonotha macrophylla* and *Tieghemella heckelii* extracts exhibited antitrypanosomal activity, but complete elimination was also not demonstrated. Although, compounds extracted from *Hyptis suaveolens* are yet to be tested against *Trypanosoma* species but diterpenoid compounds extracted from the plant when tested against *Plasmodium falciparum* were active against the parasite which showed the plants possess other antiparasitic activities. However, Ngozi (2014) and Mishra (2021) identified the presence of alkaloids, tannins, and saponins in *Hyptis suaveolens*, which are known for their antimicrobial properties. This suggests that *Hyptis suaveolens*, while potentially not a standalone treatment for trypanosomiasis, could be explored for use in combination with other plant extracts for a more efficacious therapeutic approach. In another study, Madaki (2016) found that *Hyptis suaveolens*, along with other plant

extracts, demonstrated significant in vitro antitrypanosomal activity. There may be other trypanocidal compounds within *Hyptis suaveolens* that have not yet been isolated and tested.

For the other disease studied, Leishmaniasis, the study identified Pheophytin A and α & β amyrin, as the most effective compounds when compared to other isolated compounds against *Leishmania major*, a parasite causing leishmaniasis. Pheophytin A was found to be more effective against a different Leishmania species, *Leishmania major*, compared to Pheophytin B. This is in contrast with the study by López-Arencibia (2020), who reported that Pheophytin B was more active against yet another *Leishmania* species. Recent studies have also identified other compounds isolated from *Hyptis* species as a potent compound against *Leishmania major*, the parasite causing leishmaniasis (Guimarães, 2009; Falcao, 2013; Shamsi, 2018; Sijm, 2019; Bano, 2022). Our finding reported pheophytin B as the compound with the poorest activity against *L. major*. However, Mishra (2023) demonstrated that Pheophytin B, when combined with Lipid-based nano drug delivery systems, holds considerable promise in therapeutic intervention for leishmaniasis by enhancing drug solubility and targeted delivery, thereby increasing its anti-leishmanial activity in vitro, suggesting potential for improved delivery methods. The findings of Mishra (2023) can be considered in subsequent research through the combination of pheophytin B with lipid-based nano drug systems or other compounds to enhance the drug sensitivity potential of the compound.

This research highlights the potential of phytosterols extracted from *Hyptis suaveolens* for treating leishmaniasis. Only α & β amyrin demonstrated moderate activity against *L. mexicana*. Alpha and beta amyrins are categorized as 4,4- dimethylsterol which is a class of phytosterol. Phytosterols which exist in all plant cell membranes are especially enriched in vegetable oils and fats, cereals, and cereal products, vegetables, fruits, and berries (Pironen and Lampi, 2004). Our finding is similar to Oaikhena et al., 2024 who reported in their research high efficacy of suaveolol and suaveolic acid (SSA) in a combination of 70:30 ratio as more effective than their its single compounds. Anyam *et al*., (2021) report on PAN-76 and HEAN-18 which contain various amounts of Compounds 16, 19-dihydroxycassa-12-en-15-one (Sandynone, 1), (5S, 7R, 8R, 9R, 10S, 13Z, 17S)-7,8:7,17:16,17-triepoxy-7,8-seco-cassa-13-ene (niloticane B, 2), (5S,7R,8R,9R,10S) -(-)-7,8-seco-7, 8-oxacassa-13,15-diene-7,17-diol (3), and (5S,7R,8R,9R,10S) -(-)-7,8-seco-7, 8-oxacassa-13,15-dien-7-ol (5) that demonstrated moderate antileishmanial activity against *in vitro Leishmania* cell lines. The synergistic effect displayed by both compounds on tested pathogens might have been attributed to the fact that they are likely to target the same biochemical pathways and/or uptake mechanisms (Lüscher et al., 2007).

The consistently poor anti-protozoal activity of α Amyrin, β Amyrin, and Pheophytin B suggests that a systematic investigation of the structure-activity relationship could yield compounds that can substantially improve the efficacy of these compounds against parasites kinetoplastids (James, 2022). The moderate antitrypanosomal and antileishmanial activities of Pheophytin A and α and β amyrin are particularly encouraging. However, scientists should explore whether combination of different phytosterols from *Hyptis suaveolens*, or even combinations with existing drugs, might increase its potency than any single compound, based on our research findings.

4.0 Conclusion

In conclusion, this study demonstrates the potential of *Hyptis suaveolens* derived phytosterols, particularly Pheophytin A and α and β Amyrin, for the treatment of trypanosomiasis and leishmaniasis. Pheophytin A and α and β Amyrin exhibited moderate *in vitro* activity against *Trypanosoma brucei brucei* and *Leishmania major* while only α and β Amyrin demonstrated moderate *Leishmania mexicana* activity compared to the established drug pentamidine. However, further research is warranted to explore the *in vivo* efficacy and safety of these phytosterols. Additionally, investigating the mechanism of action and potential synergistic effects with other compounds is crucial for their development as viable drug agents. Although the leishmanial and trypanocidal activities of the isolated phytosterols were modest, further exploration of *Hyptis suaveolens* for this purpose cannot be excluded. This study contributes to the

ongoing search for novel therapeutic strategies against neglected tropical diseases using natural products and the exploration of the use of a combination of compounds.

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