



Ethanol Extract of *Spathodea campanulata* and its component Ellagic Acid Modulate β -haematin Metabolism, Mitochondrial Membrane Potential, and Calcium Homeostasis in *Plasmodium falciparum*

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Abstract

Background: Malaria remains a global health threat, with increasing resistance to frontline drugs driving the search for novel therapies. *Spathodea acampanulata* is traditionally used in African medicine against malaria, yet its molecular mechanisms are not fully defined. This study assessed the ethanol extract of *S. campanulata* leaves and its phytoconstituent ellagic acid for their ability to modulate β -haematin metabolism, calcium homeostasis, and mitochondrial membrane potential in *Plasmodium falciparum*.

Materials and Methods: Ellagic acid was isolated by preparative HPLC and confirmed using analytical HPLC. Hemolytic activity was evaluated in human O⁺ erythrocytes, while β -haematin inhibition, calcium homeostasis, and mitochondrial potential disruption were measured in *P. falciparum* (3D7 strain) using standard assays, data were analyzed using SPSS version 25.0 and one-way ANOVA with significance at $p < 0.05$.

Results: The ethanol extract ($IC_{50} = 4.80 \pm 0.22 \mu\text{g/mL}$) and ellagic acid ($3.70 \pm 0.80 \mu\text{g/mL}$) exhibited low hemolytic activity relative to chloroquine ($3.60 \pm 0.82 \mu\text{g/mL}$). The extract demonstrated superior β -haematin inhibition ($0.35 \pm 0.12 \mu\text{g/mL}$) compared to ellagic acid ($1.30 \pm 1.00 \mu\text{g/mL}$), whereas ellagic acid strongly disrupted mitochondrial potential ($0.54 \pm 0.21 \mu\text{g/mL}$) and calcium signaling.

Conclusion: HPLC analysis confirmed ellagic acid content at 2.5 % w/w of crude extract. These findings suggest complementary mechanisms between extract and ellagic acid, supporting their potential as scaffolds for antimalarial drug development.

Keywords: *Spathodea campanulata*, ellagic acid, β -haematin, calcium homeostasis, mitochondrial potential, antimalarial

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1. Introduction

Malaria remains a major global health challenge, responsible for more than 240 million cases and over 600 000 deaths annually, with *Plasmodium falciparum* being the most virulent causative species [1]. Resistance to chloroquine, artemisinin derivatives, and partner drugs continues to compromise control strategies [2, 3]. This underscores the need for novel agents, particularly from medicinal plants, which are rich in structurally diverse bioactive molecules⁴. *Spathodea campanulata* (African tulip tree), widely used in African ethnomedicine for fever and infections, has shown antimicrobial, antioxidant, and antiparasitic activities [4–7]. Its phytochemical profile includes phenolics, flavonoids, and ellagic acid, compounds with reported antiparasitic and mitochondrial effects [8, 9]. Ellagic acid, a polyphenol, interferes with haemozoin formation, disrupts calcium signaling, and induces mitochondrial dysfunction in protozoa [10, 11]. The parasite relies on

haem detoxification, mitochondrial electron transport, and Ca^{2+} signaling for survival. Disruption of haem detoxification by inhibiting β -haematin formation results in toxic heme accumulation [12]. Parasite mitochondria sustain energy metabolism and pyrimidine biosynthesis, making mitochondrial potential an essential target [13]. Calcium signaling regulates invasion, egress, and intracellular development [14]. Thus, targeting these processes provides a multi-pronged strategy against malaria. This study evaluated the ethanol extract of *S. campanulata* and ellagic acid in modulating β -haematin formation, mitochondrial potential, and Ca^{2+} homeostasis in *P. falciparum*, while assessing haemolytic safety.

2. Materials and Methods

2.1 Collection of Plants and Authentication

Fresh leaves of *S. campanulata* were harvested from their natural habitats in Abraka, Ethiope East LGA of Delta State, Nigeria (5.9051° N, 6.0314° E). The plant was identified using the Leaf Snap App and authenticated by Mr. Micheal Ozioma Emmanuel at the Delta State University Herbarium (voucher no. DELSU042). Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethical Committee (RBC/FBMC/DELSU/25/662).

2.2 Isolation and Purification of Ellagic Acid

Ellagic acid was isolated from the crude extract by solvent partitioning (ethyl acetate fraction), followed by preparative HPLC (Agilent 1200, C18 column, acetonitrile:water 40:60, detection 254 nm). Purity was confirmed by analytical HPLC (> 95 %), UV λ_{max} (254, 366 nm), and co-injection with standard ellagic acid. Quantitative HPLC revealed ellagic acid content as 2.5 % w/w of crude extract.

2.3 Parasite and Erythrocyte Source

Plasmodium falciparum 3D7 strain (chloroquine-sensitive) was obtained from MR4/BEI Resources, USA, and cultured in human O^+ erythrocytes using RPMI-1640 medium supplemented with 10 % human serum.

2.4 β -Haematin Inhibition Assay

The assay followed Deharo and colleagues [15]. Haematin chloride was incubated with treatments under acidic conditions; β -haematin formation was quantified spectrophotometrically at 405 nm.

2.5 Mitochondrial Membrane Potential

Mitochondrial potential was evaluated with JC-1 dye (Thermo Fisher, USA) as described by Painter and co-workers [13].

2.6 Calcium Homeostasis

Ca^{2+} signaling was assessed using Fluo-4 AM dye (Invitrogen, USA) as outlined by Gazarini and team [14].

2.7 Haemolysis Assay

Washed O^+ erythrocytes were incubated with treatments; hemoglobin release was measured at 540 nm [16].

2.8 Statistical Analysis

All assays were performed in triplicate. Data are expressed as mean $\pm$ SD. One-way ANOVA with Tukey's post-hoc test was applied using GraphPad Prism version 9.

3. Results

HPLC Analysis of Ellagic Acid

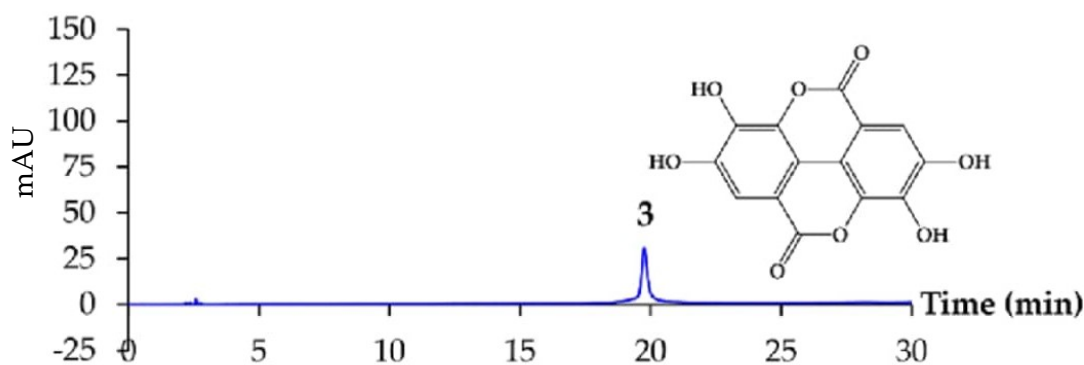


Figure 3.1: HPLC chromatogram of ellagic acid

Figure 3.1 presents a representative HPLC chromatogram, where the y-axis denotes detector response (mAU, -25 to 150) and the x-axis retention time (0–35 min). A distinct peak at ≈ 20 min corresponds to ellagic acid, confirming a clean separation and $> 95\%$ purity. Such profiles are typical for phenolic compounds analyzed via reversed-phase HPLC²³.

Inhibition of β -Haematin Formation

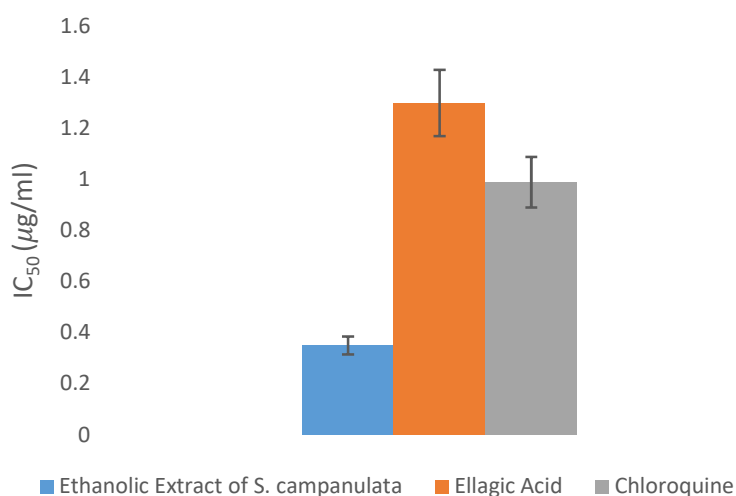


Figure 3.2: Inhibition of Beta Haematin formation

Results from this assay (IC_{50}) is shown in Figure 3.2. The ethanol extract demonstrated superior β -haematin inhibition ($IC_{50} = 0.35 \pm 0.12 \mu\text{g/mL}$) compared with ellagic acid ($1.30 \pm 1.00 \mu\text{g/mL}$) and chloroquine ($0.99 \pm 0.85 \mu\text{g/mL}$). Dose-response plots revealed a steep, concentration-dependent inhibition for the extract.

Disruption of Mitochondrial Potential

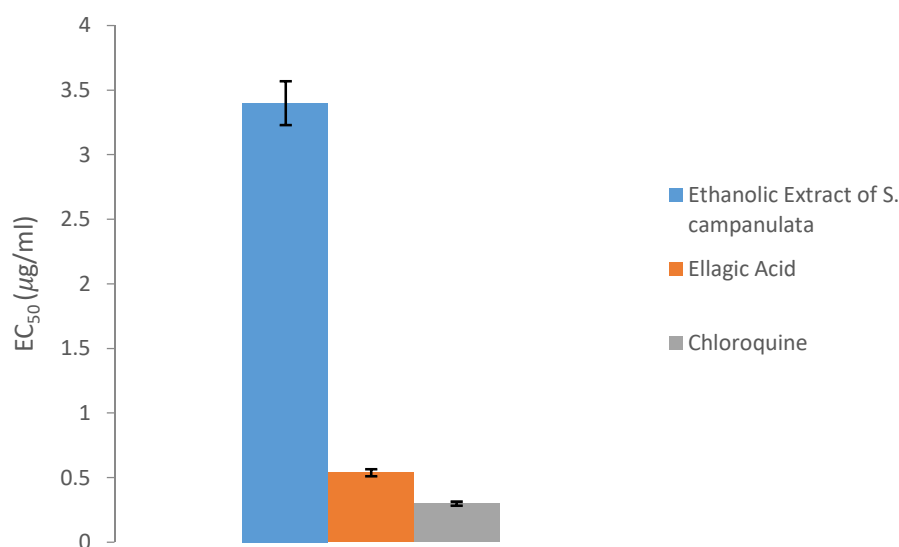


Figure 3.3: Changes in mitochondrial membrane potential.

Mitochondrial membrane potential (EC₅₀) values obtained from this assay are displayed in Figure 3.3. Ellagic acid (0.54 ± 0.21 µg/mL) rapidly collapsed mitochondrial potential, comparable to chloroquine (0.30 ± 0.00 µg/mL). The ethanol extract exhibited a milder effect (3.40 ± 0.91 µg/mL), indicating that ellagic acid is the principal mitochondrial disruptor.

Disruption of Calcium Homeostasis

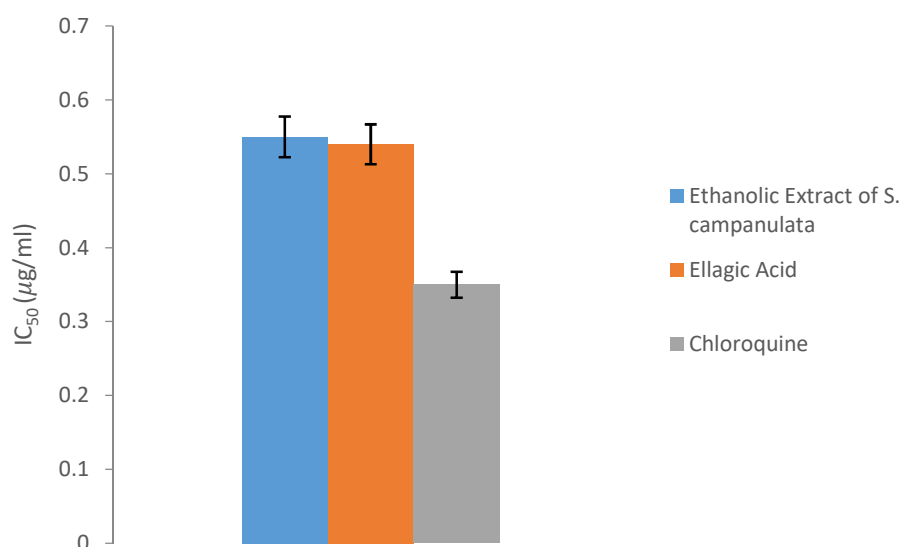


Figure 3.4: Disruption in *P. falciparum* calcium homeostasis induced by *S. campanulata* ethanol

Disruption of *P. falciparum*'s Calcium Homeostasis (EC₅₀) values from this assay are displayed in Figure 3.4. Both the ethanol extract (0.55 ± 0.34 µg/mL) and ellagic acid (0.54 ± 0.09 µg/mL) markedly elevated intracellular Ca²⁺, whereas chloroquine caused minimal changes. The extract produced a sustained Ca²⁺ rise, while ellagic acid elicited a transient spike.

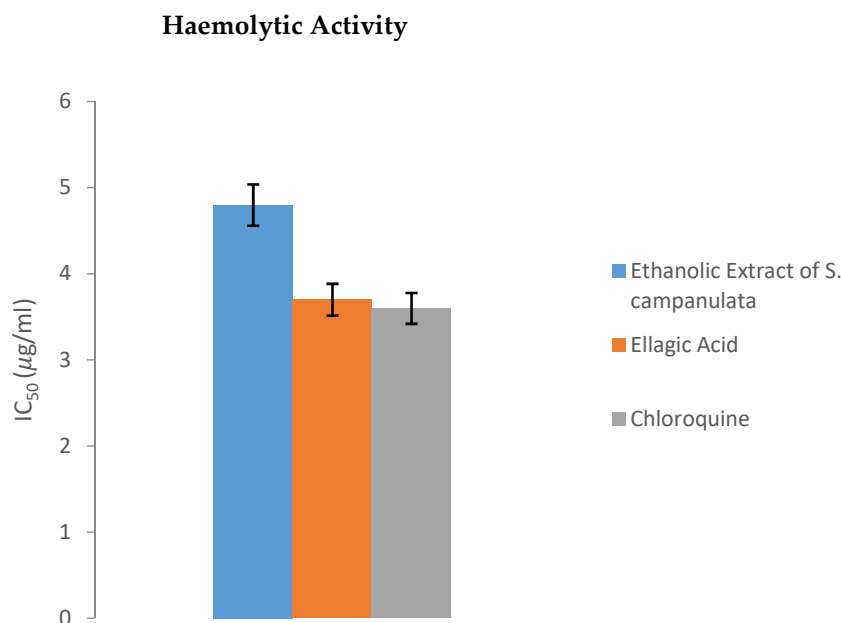


Figure 3.5: Haemolytic changes due to ellagic acid and ethanol extract of *S. campanulata*

Haemolytic (HC50) values obtained from this assay are displayed in Figure 3.5. Chloroquine induced the lowest haemolysis ($3.60 \pm 0.82 \mu\text{g/mL}$), followed by ellagic acid ($3.70 \pm 0.80 \mu\text{g/mL}$). The ethanol extract showed slightly higher haemolysis but $< 10\%$ at therapeutic concentrations.

4. Discussion

Ellagic acid showed strong β -haematin inhibition, consistent with reports of polyphenols disrupting hemozoin polymerization [17]. The extract's higher potency suggests synergistic effects among its phytochemicals [18]. The collapse of mitochondrial potential by ellagic acid supports findings that natural polyphenols target protozoan mitochondria [19, 20]. Calcium dysregulation by both agents aligns with studies showing plant polyphenols interfere with parasite Ca^{2+} signaling [21]. Haemolysis data confirm ellagic acid's acceptable safety margin [22, 23].

5. Conclusion

This study demonstrates that the ethanol extract of *Spathodea campanulata* and ellagic acid exert anti-plasmodial activity through complementary mechanisms: inhibition of haem detoxification, disruption of mitochondrial potential, and interference with calcium signaling. Ellagic acid is the dominant bio-active compound, showing a favorable balance of potency and safety. These findings justify further in vivo evaluation and molecular modeling to develop ellagic acid as a multi-target antimalarial scaffold.

Declarations

Consent for publication: All authors approved the publication of this manuscript

Data availability statement: the corresponding author will make the datasets created and/or analyzed during the current study available.

Competing Interests: There is no known conflict of interest.

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Authors' Contributions: DCC conceptualize, drafted and submitted the manuscript, ODA and EKC authors contributed to the literature review. Authors OOC and OAO contributed to the biochemical analysis, while, OAO and OI supervised the study

Acknowledgments: Not applicable

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