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Phytochemical Analysis, Antimalarial and Antioxidant Activities of *Musa Paradisiaca* Rhizome

Maryam Abouelfetouh[1]*, Zain Elaabedeen Metwaly[1], El-Sayed Mahdy Marwan[1], Zulfiqar Ali[2], Ikhlas A. Khan[2], El-Sayed Shaker Mansour[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*
[2]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

***Corresponding author:** Maryam Abouelfetouh, Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt. Tel. +201557889985*
E-mail address: maryamaboelfetouh@mans.edu.eg

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ABSTRACT

Objectives: In this study, *Musa paradisiaca* rhizome was investigated for its anti-malarial, and antioxidant activities in addition to the phytochemical analysis. This investigation is motivated by the need for new antimalarial agents and the potential utilization of banana plant bio-waste. **Methods**: *M. paradisiaca* rhizomes were collected, authenticated, and stored following established guidelines. After being air-dried and powdered, the rhizomes were extracted with 70% methanol, then fractionated, and compounds were isolated and purified using silica gel column chromatography, preparative TLC, and repeated crystallization. An *in-vitro* antimalarial assay was conducted using the pLDH assay protocol against *P. falciparum* D6 and W2 strains. The antioxidant activity was evaluated using the ABTS·+ radical cation decolorization assay, with IC_{50} values compared to *L*-ascorbic acid. Total phenolic content was determined using the Folin-Ciocalteu method, with gallic acid as the standard. **Results**: Seven compounds were isolated from the rhizome, based on spectral data (NMR and MS), they were identified as 31-norcyclolaudenone (**1**), a mixture of *β*-sitosterol and stigmasterol (**2**), 31-norcyclolaudenol with its regio-isomer (**3**), *β*-sitosterol glucoside (**4**), indole-3-carboxaldehyde (**5**), methyl 3,4-dihydroxybenzoate (**6**), and adenosine (**7**). This is the first report to indicate the isolation of compounds **5**, **6** and **7** from the family Musaceae. The petroleum ether extract exhibited significant inhibition of *P. falciparum* growth, with IC_{50} values of 18725.9 ng/mL and 15244.5 ng/mL for strains D6 and W2, respectively, along with high selectivity indices towards the parasite (>2.5 for D6 and >3.1 for W2). The ethyl acetate extract showed moderate antioxidant activity with an IC_{50} value of 48.25 ± 0.23 µg/mL in the ABTS assay. The total phenolic content was moderate, measured at 104.9767 mg/g (GAE). **Conclusion:** *M. paradisiaca* rhizomes are a promising source of bioactive compounds with significant antimalarial and antioxidant activities. These findings support the potential therapeutic applications of banana plant extracts and underscore the value of natural products in drug discovery.

***Keywords**: Anti-malarial, antioxidant, phenolic, Musa paradisiaca, adenosine, In-vitro.*

INTRODUCTION

Herbal medicine has played a crucial role in shaping modern medicine and the discovery of new drugs. Its extensive historical use in addressing various health issues has laid the groundwork for the development of therapeutic agents [1]. *Musa paradisiaca*, commonly known as the banana, belongs to the Musaceae family. It is a compact family that includes three genera: *Musa*, *Ensete*, and *Musella*, encompassing a total of 41 species [2]. *M. paradisiaca* Linn. is an herbaceous plant that features a strong tree-like pseudo stem, and a crown of large elongated oval deep-green leaves, with a distinct midrib. Each plant produces a singular inflorescence-like drooping spike, characterized by large, dark red, concave, ovate bracts, and somewhat fleshy [3]. The oblong fruits, measure 5-7 cm in length in the wild form and longer in cultivated varieties [4]. Notably, bananas rank among the world's most important fruit crops, contributing significantly to the global annual production of food. However, a substantial portion of the banana plant, including elements like rhizomes and roots, is often discarded as waste, giving rise to environmental concerns and presenting economic opportunities that remain undiscovered. Considering the extensive traditional medicinal use and the substantial amount of bio-waste produced, there is a compelling argument to explore the potential economic and medicinal advantages offered by the biomass of discarded banana plant material. Additionally, thorough phytochemical screening has revealed the existence of diverse bioactive compounds, such as alkaloids, flavonoids, phenols, saponins, tannins, and vitamins (A, B, and C) in banana plants [5,6]. The historical applications of banana leaves, stem juice, and fruit involve the treatment of various conditions such as eczema, burns, diarrhea, dysentery, cholera, otalgia, and hemoptysis [7]. The banana plant's root is utilized as an anthelmintic and for addressing issues related to blood disorders and venereal diseases [8]. Additionally, the unripe fruit is employed in managing diverse health conditions, including diabetes, uremia, nephritis, gout, hypertension, and cardiac disease [9].

Malaria, a disease with a centuries-long history, continues to impact people worldwide, causing over 200 million clinical cases annually [10]. Although some developed nations have successfully eliminated the disease, its persistence in tropical regions presents a real global health challenge. Malaria arises from the infection of parasites belonging to the *Plasmodium* genus with *Plasmodium falciparum* being the most common [11]. Despite earlier optimistic projections suggesting the potential eradication of malaria by 2030 [12], there has been a consistent rise in case numbers over the past few years. In 2021 alone, there were approximately 247 million reported malaria cases and 619,000 malaria-related deaths. This represents an increase of 33 million cases and 181,000 deaths compared to the figures reported in 2015 [13]. Various factors may contribute to this, including biological aspects such as the development of resistance in parasites and mosquito vectors to drugs and insecticides, environmental influences like climate change, and alterations in vector distribution. Addressing the global challenge of resistance to antimalarial drugs is a significant issue. It complicates the treatment and management of diseases, emphasizing the urgent need for improved drugs that are effective and don't pose complications. Developing these new treatments is crucial not only for preventing diseases like malaria but also for ensuring their effective and reliable treatment.

Antioxidants have sparked scientific interest for their numerous advantages, including anti-aging and anti-inflammatory properties. These compounds, found in various groups, work to counteract free radicals and reactive oxygen species (ROS) within the cell [14]. Natural antioxidants, typically sourced from plants, exhibit varying levels of activity influenced by factors such as plant species, diversity, extraction or processing methods, and growing conditions [15].

This paper looks into the crucial search for new drugs from natural sources such as *M. paradisiaca* rhizomes, highlighting their potential to change how we treat malaria and oxidative stress while minimizing the side effects of the current drugs.

This study aims to investigate the antimalarial and antioxidant activities of *Musa paradisiaca* rhizome, alongside conducting a phytochemical analysis. This research is driven by the necessity for new antimalarial agents and the potential to utilize banana plant bio-waste in therapeutic applications.

MATERIAL AND METHODS

General experimental procedures

The solvents used for extraction, chromatographic separation, and crystallization (petroleum ether, *n*-hexane, methylene chloride, ethyl acetate, *n*-butanol, and methanol) were of analytical grade, obtained from El Nasr Company for Pharmaceutical Chemicals (Egypt) and Fisher-Johns Scientific Company (USA). Analytical thin-layer chromatography (TLC) was conducted using silica gel 60 GF_{254} (20 x 20 cm, 0.2 mm thickness) on aluminum sheets and precoated RP-C18 F_{254} plates (20 x 20 cm, 0.2 mm thickness) on aluminum sheets from Merck Co. (Darmstadt, Germany). Preparative TLC was carried out on preparative layer plates prepared using 100 gm silica gel GF_{254} from Merck (Germany). The visualization of compounds on TLC chromatograms was achieved using vanillin-sulfuric acid spray (prepared with 5% H_2SO_4 in ethanol). Normal phase column chromatography (CC)

was performed using silica gel 60-230 mesh size from Merck (Germany), while reversed-phase chromatography was done using RP-C18 from Merck (Germany). For the ABTS antioxidant assay, ABTS (Sigma-Aldrich Chemicals, St. Louis, USA), potassium persulfate (Sigma-Aldrich Chemical Company, St. Louis, USA), *L*-ascorbic acid, and pure ethanol (ElNasr Co. for Pharmaceutical Chemicals, Egypt) were used. The Folin–Ciocalteu reagent was purchased from Sigma-Aldrich Chemicals, St. Louis, USA, while sodium carbonate and gallic acid hydrate were sourced from ElNasr Co. for Pharmaceutical Chemicals (Egypt). The *in vitro* antimalarial assay used DMSO, Malstat™ reagent, and RPMI-1640 medium suitable for cell culture obtained from Sigma-Aldrich (USA), with NBT (nitroblue tetrazolium), PES (phenazine ethosulfate), and all other chemicals purchased from Sigma-Aldrich (USA), The rotary flash evaporator used was from Buchi (Switzerland). The melting point apparatus was from Fisher-Johns Scientific Co. (USA). The UV lamp (254 and 366 nm) was from Desaga (Germany), and the digital balance was from Sartorius (Germany). NMR spectra were obtained on a Bruker AU III 400 MHz NMR spectrometer using DMSO-d_6, CD_3OD, or $CDCl_3$ solvents. An Agilent Technologies 6200 series mass spectrometer was used to record mass spectra.

Cell culture

Laboratory strains of *Plasmodium falciparum*, chloroquine-sensitive D6, and chloroquine-resistant W2 strains, were sourced from the Malaria Research and Reference Reagent Resource Center in the United States (MR4). The D6 strain of *P. falciparum*, originally collected in Sierra Leone, is recognized for its sensitivity to drugs. On the other hand, the W2 strain was cloned from the Indochina III/CDC isolate, derived from a patient in Laos who did not respond to chloroquine treatment. The W2 strain is known to be resistant to chloroquine but responds to mefloquine. These *P. falciparum* strains were cultured using A+ human red blood cells, which were obtained from the Mississippi Blood Services. The cultures were maintained at a 5% hematocrit level in a complete malaria culture medium (RPMI 1640) supplemented with 60 µg/mL amikacin, 2.2 g/L sodium bicarbonate, and 25 mM HEPES buffer. The medium was changed every 48 hours, and the cultures were kept at 37°C in an incubator with a controlled atmosphere of 90% nitrogen, 5% carbon dioxide, and 5% oxygen.

Plant material

The rhizomes of *Musa paradisiaca* Linn. were gathered from the Faculty of Pharmacy Farm, Mansoura University, Egypt, in August 2021. The collection of plant material was carried out by established ethical and legal guidelines. Prof. Ibrahim Mashaly, Professor of Ecology at the Faculty of Science, Mansoura University, Egypt, authenticated the plant material to ensure its taxonomic identity. The plant material was harvested during the flowering stage to ensure optimal chemical composition. After collection, it was immediately transported to the laboratory and subjected to proper drying and storage procedures to maintain its integrity and prevent degradation. The plant's voucher specimens have been stored in the Pharmacognosy Department of the Faculty of Pharmacy at Mansoura University in Egypt, and they have been assigned specific codes (MM-3-2021).

Preparation of the extracts and isolation of the compounds:

The rhizomes of *M. paradisiaca* were first air-dried in the shade and subsequently ground into a fine powder. The extraction process involved macerating 1250 g of the powdered, air-dried parts in 70% methanol at room temperature using a total of ten liters of methanol, split into seven cycles. Afterward, the collected methanolic extract was subjected to evaporation under reduced pressure and allowed to dry within a desiccator containing anhydrous $CaCl_2$. This process resulted in the formation of a dark brown, viscous residue weighing 112.4 g. The dried methanolic residue was then dissolved in the smallest possible volume of methanol. It was subsequently diluted with an appropriate volume of distilled water and subjected to fractionation using petroleum ether, methylene chloride, ethyl acetate, and *n*-butanol. In each instance, the solvent was dried under reduced pressure, resulting in five distinct fractions: a petroleum ether-soluble fraction weighing 37 g (fraction A), a methylene chloride-soluble fraction weighing 14 g (fraction B), an ethyl acetate-soluble fraction weighing 11 g (fraction C), a *n*-butanol-soluble fraction weighing 20 g (fraction D), and an aqueous fraction weighing 27 g (fraction E).

Fraction A experienced silica gel column chromatography and underwent elution using hexane – DCM gradient (50-100%) and then DCM – MeOH (0-90%). The fractions sharing similar R_f values were combined and subsequently purified through chromatography on silica gel columns. The purification process also involved repeated crystallization resulting in compounds **1, 2, 3,** and **4**.

Fraction B was subjected to silica gel column chromatography and eluted with DCM – MeOH (0-95%). Collected fractions were combined based on similar R_f values, and subjected to purification through column chromatography, PTLC, and repeated crystallization yielding compounds **5** and **6**.

Fraction D was subjected to reversed phase chromatography using C-18 silica gel column and eluted using H_2O with increasing amounts of MeOH (from 100% H_2O to 100% MeOH), Combining fractions with

similar R_f values, they were subsequently purified using chromatography on silica gel columns. The purification procedure also included multiple rounds of crystallization resulting in the isolation of compound **7**.

Total phenolic assay using the Folin-Ciocalteu method

Total phenolic were quantified using the Folin-Ciocalteu colorimetric method, with gallic acid as the standard. As mentioned in the publication by Genwali [16]. To begin, one milligram of the total methanolic extract (prepared in 70 % methanol) of *M. paradisiaca* was dissolved in 1 mL of MeOH. Subsequently, 4 mL of sodium carbonate solution (7% w/v) was added and thoroughly mixed with 5 mL of Folin-Ciocalteau reagent (10% v/v). The resulting blue mixture was incubated for 30 minutes in a water bath at 40°C. Following incubation, the absorbance at 760 nm was measured against a blank solution. The blank consisted of 1 mL of methanol mixed with 4 mL of sodium carbonate solution (7% w/v) and 5 mL of Folin-Ciocalteau reagent (10% v/v), resulting in a final volume of 10 mL. Each measurement was performed in triplicate to ensure accuracy. To construct the calibration curve for quantifying phenolic content, absorbance values were obtained at various concentrations of gallic acid ranging from 0 to 100 µg/mL. These gallic acid solutions were prepared using the same method described above. Finally, the phenolic content was expressed as milligrams per gram of gallic acid equivalent (GAE).

***In-vitro* anti-malarial assay**

It was conducted based on the parasite lactate dehydrogenase (pLDH) assay protocol [17]. The activity was evaluated in two *P. falciparum* clones: Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant) sourced from the Malaria Research and Reference Reagent Resource Center in the United States (MR4). Vero cells (kidney epithelial cells from an African green monkey) were used to assess cytotoxicity. The assay was focused on assessing the influence of the fractions on the growth of asynchronous cultures of *P. falciparum*. Various dilutions of the fractions were prepared in either DMSO or RPMI-1640 medium (2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate) were added to *P. falciparum* cultures with 2% hematocrit and 2% parasitemia in clear flat-bottomed 96-well plates. In these wells, 10 µL of test samples, at varying concentrations, were introduced. The 96-well plate was then placed in a Billups-Rothenberg MIC-101 Modular Incubator Chamber, where it was exposed to a gas mixture of 90% N_2, 5% O_2, and 5% CO_2, and maintained at 37 °C for 72 hours.

Parasitic LDH activity was determined using the Malstat™ reagent (obtained from Sigma-Aldrich Chemical Company, St. Louis, USA) consisting of: {sodium lactate 4 g, Tris buffer 1.32 g, triton X-100 400 µL, and 3-acetylpyridine adenine dinucleotide APAD 0.02 g in 200 mL of distilled water}. I20 µL of the incubation mixture was combined with 100 µL of the Malstat™ reagent and left to incubate at room temperature for 30 minutes. Subsequently, 20 µL of a 1:1 mixture of NBT/PES (nitrotetrazolium blue chloride/ phenazine ethosulphate) (purchased from Sigma-Aldrich Chemical Company, St. Louis, USA) was added, and the plate was further incubated in darkness for 1 hour. The reaction was terminated by adding 100 µL of a 5% acetic acid solution. The plate's absorbance was measured at 650 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments Co.) and IC_{50} values were computed. Selectivity Index (SI) = IC_{50} (Vero Cells)/IC_{50} (*P. falciparum*) and the criteria of the selectivity index range were delineated in a previous study [18].

Antioxidant activity by ABTS assay

The ABTS·+ radical cation assay was conducted following the protocol by Re et al. [19]. The ABTS·+ radical cation was prepared by mixing equal volumes of ABTS stock solution (7 mM in distilled water) and potassium persulfate solution (3.5 mM in distilled water) at a 2:1 ratio. This mixture was left in the dark at room temperature for 12-16 hours. The resulting ABTS·+ solution was diluted with ethanol to an absorbance of 0.7±0.02 at 734 nm and equilibrated at 30 °C. For the assay, 1.5 mL of the ABTS·+ working solution was mixed with 10 µL of the test fractions at various concentrations (10-100 µM). The absorbance was measured immediately, at 0.5, 1 minute, and then at 5-minute intervals until a steady state (15 minutes) was achieved. The absorbance for each test fraction (A_test) was recorded at 15 minutes. All measurements were performed in triplicate. The percent inhibition was calculated using the equation: Percent Inhibition=100× (A blank−A test)/A blank. Where A blank is the absorbance of the ABTS·+ radical cation in H2O and EtOH directly before reaction time (0 min), and A test or is the absorbance of the reaction mixture at reaction time= 15 min after adding the test fraction

To measure the IC_{50} value, the percent inhibition values for different concentrations of the test fraction were plotted against the logarithm of the concentrations. A dose-response curve was generated, and the IC_{50} values (the concentration needed to reduce ABTS·+ absorbance by 50% at 734 nm) were obtained through extrapolation from regression analysis. The IC_{50} values of the test fractions were compared to *L*-ascorbic acid, the standard antioxidant. A lower IC_{50} indicates higher antioxidant potency.

Statistical Analysis

The antimalarial and antioxidant activities of different fractions obtained from *M. paradisiaca* extracts were statistically analyzed to determine their efficacy and selectivity. The data were expressed as mean ± standard deviation (SD) from three independent experiments.

Antimalarial Activity

The half-maximal inhibitory concentration (IC_{50}) values and selectivity indices (SI) for each fraction were calculated using non-linear regression analysis in STATA 17 software (StataCorp LLC, College Station, TX). IC_{50} values were determined for *P. falciparum* D6 and W2 strains, and cytotoxicity against Vero cells was evaluated to determine the safety profile of each fraction. The selectivity index was calculated as the ratio of the IC_{50} value for Vero cells to the IC_{50} value for the *P. falciparum* strains, with a higher SI indicating greater selectivity towards the malaria parasite compared to the Vero cells. The therapeutic window of the fractions was assessed by calculating selectivity indices, and antimalarial activity was compared among different fractions using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, with statistical significance set at $p < 0.05$.

Antioxidant Activity

The percentage inhibition of ABTS radicals at different concentrations (10, 20, 40, 60, 80, and 100 µg/mL) was calculated for each fraction. The IC_{50} values, representing the concentration required to inhibit 50% of the ABTS radicals, were calculated using non-linear regression analysis in STATA 17 software (StataCorp LLC, College Station, TX). The antioxidant activities of the fractions were compared using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons to identify significant differences among the extracts. *L*-ascorbic acid was used as a reference standard for comparison. Statistical significance was set at $p < 0.05$.

All statistical analyses were performed using STATA 17 software (StataCorp LLC, College Station, TX). Data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of the Isolated Compounds

The compounds isolated from different extracts obtained from 70% MeOH extract of *M. paradisiaca* are seven (**Figure 1**) they were identified based on a comparison of their spectral data with those reported in the literature. 31-norcyclolaudenone (**1**): The spectral data was in full agreement with those published by Knapp et al [20] from the isolation of the same compound in the same family.

A mixture of *β*-sitosterol and stigmasterol (**2**) [21]: these compounds had been previously isolated from this plant [22].

31-Norcyclolaudenol (**3**): the spectral data was in full agreement with those previously published [23] from the isolation of the same compound in the same family.

β-Sitosterol-3-*O*-*β*-D-glucoside (**4**) was identified by co-chromatography using an authentic sample of *β*-sitosterol-3-*O*-*β*-D-glucoside previously isolated at the Pharmacognosy Department, Mansoura University. It has been previously isolated from *M. paradisiaca* [24]. Indole-3-carboxaldehyde (**5**) [25], methyl 3,4-dihydroxybenzoate (**6**) [26], and adenosine (**7**) [27] were confirmed by comparing their NMR data with reported ones. Compounds **5, 6** and **7** are isolated from the family Musaceae for the first time.

Total phenolic assay by Folin Ciocalteau

At a concentration of 1mg/mL, the *Musa paradisiaca* methanolic extract exhibited an absorbance value of 0.8, and by establishing a calibration curve with different concentrations of gallic acid (Figure 2) and the application of the equation $y = 0.0086x - 0.1028$. The calculated phenolic content was 104.9767 mg /g (GAE). So, the plant possesses moderate phenolic content, this finding can have implications for the potential use of *M. paradisiaca* extract in various applications, as phenolic compounds are known for their antioxidant properties and potential health benefits.

Antimalarial activity of *M. paradisiaca* fractions

The results of the antimalarial activity testing of different fractions obtained from *M. paradisiaca* extracts are summarized in **Table 1**. The fractions include total extract, petroleum ether fraction, methylene chloride fraction, ethyl acetate fraction, and *n*-butanol fraction. The antimalarial activity was evaluated against *P. falciparum* D6 and W2 strains, and Vero cells were used as a reference for cytotoxicity. The IC_{50} values (half-maximal inhibitory concentration) and selectivity indices (SI) were determined for each fraction.

Notably, the total extract demonstrated considerable antimalarial activity against both *P. falciparum* D6 and W2 strains, with IC_{50} values of 31885.1 ng/mL and 15364.8 ng/mL, respectively. The selectivity indices (SI) for this extract were greater than 1.5 and 3.1 for D6 and W2, respectively, suggesting its potential as a source of antimalarial drug. Moreover, the total extract exhibited a high IC_{50} value against Vero cells, exceeding 47600 ng/mL. The petroleum fraction also exhibited promising antimalarial activity with IC_{50} values of 18725.9 ng/mL and 15244.5 ng/mL for *P. falciparum* D6 and W2, respectively. The selectivity

Table 1. Antimalarial assay results.

Tested fraction	*P. falciparum* D6 IC_{50} (ng/mL) ± SD	*P. falciparum* D6 SI	*P. falciparum* W2 IC_{50} (ng/mL) ± SD	*P. falciparum* W2 SI	Vero IC_{50} (ng/mL)	Test concentration
Total extract	31885.1 ± 180.2	>1.5	15364.8 ± 105.4	>3.1	>47600	47600-5288.9 ng/mL
Petroleum ether	18725.9 ± 125.7	>2.5	15244.5 ± 105.4	>3.1	>47600	47600-5288.9 ng/mL
Methylene chloride	36917.6 ± 200.1	>1.3	>47600	1	>47600	47600-5288.9 ng/mL
Ethyl acetate	>47600	1	>47600	1	>47600	47600-5288.9 ng/mL
n-Butanol	>47600	1	>47600	1	>47600	47600-5288.9 ng/mL

indices for this fraction were greater than 2.5 and 3.1, suggesting its selectivity toward the malaria parasite. The methylene chloride fraction displayed strong antimalarial activity with an IC_{50} value of 36917.6 ng/mL against *P. falciparum* D6, while its IC_{50} against W2 was greater than 47600 ng/mL. The selectivity index for D6 was greater than 1.3, indicating its potential as an antimalarial agent. In contrast, the ethyl acetate and *n*-butanol fractions exhibited IC_{50} values exceeding 47600 ng/mL against both *P. falciparum* D6 and W2, suggesting limited antimalarial activity for these fractions. The selectivity indices were 1, indicating low selectivity for the malaria parasite.

Antioxidant activity evaluation using ABTS assay

The percentage inhibition of ABTS radicals at different concentrations (10, 20, 40, 60, 80, and 100 µg/ml) and the calculated IC_{50} values are presented in **Table 2**.

EA extract from *M. paradisiaca* displayed considerable antioxidant activity, with a progressive increase in inhibition from 16.1% at 10 µg/mL to 78.1% at 100 µg/mL. The IC_{50} value for EA extract was calculated as 48.25±0.23 µg/mL, indicating a moderate antioxidant potential compared to L-ascorbic acid. and highest antioxidant activity among other fractions. The petroleum ether extract from *Musa paradisiaca* exhibited a dose-dependent increase in antioxidant activity, reaching an inhibition of 64.0% at the highest concentration. The IC_{50} value for this extract was 82.22±0.46 µg/mL, indicating a notable antioxidant effect, although lower than both Vitamin C and EA extract. DCM extract from *Musa paradisiaca* demonstrated medium antioxidant potential, with a percentage inhibition of 59.4% at 100 µg/mL. The IC_{50} value for DCM extract was 93.95±0.41 µg/mL. For the n-butanol extract from *Musa paradisiaca* the IC_{50} was calculated as 126.90±0.53 µg/mL, indicating a comparatively lower antioxidant potential compared to other extracts and L-ascorbic acid.The cumulative antioxidant activity of all extracts (Total) from *M. paradisiaca* showed a dose-dependent increase, with a percentage inhibition of 65.6% at 100 µg/mL. The calculated IC_{50} value for the total extract was 65.51±0.32 µg/mL, suggesting a collective antioxidant effect.

Results Interpretation and discussion:

The results of the antimalarial activity testing indicate that the Total Extract and Petroleum Fraction of *Musa paradisiaca* possess significant potential as sources of antimalarial compounds. Their low IC_{50} values against *P. falciparum* strains and relatively high selectivity indices suggest their effectiveness in inhibiting the growth of the malaria parasite while minimizing toxicity to Vero cells. On the other hand, the Methylene Chloride Fraction displayed promising antimalarial activity against *P. falciparum* D6 but showed limited activity against W2. In contrast, the Ethyl Acetate and Butanol Fractions exhibited negligible antimalarial activity, with IC_{50} values exceeding 47600 ng/mL for both strains. These fractions may not be suitable sources of antimalarial compounds. Overall, the results of this study provide valuable insights into the antimalarial potential of different fractions of *M. paradisiaca* extracts, leading the way for future investigations to identify and develop novel antimalarial agents.

The ABTS assay results indicate that *M. paradisiaca* extracts possess varying degrees of antioxidant activity. particularly the Ethyl acetate and the petroleum ether fractions exhibited noteworthy antioxidant potential. These findings contribute valuable insights into the potential use of *Musa paradisiaca* as a natural source of antioxidants with potential health benefits.

The correlation between the nature of the identified constituents and the observed bioactivity and the expected mechanism of action:

The seven compounds isolated from *M. paradisiaca* have been reported to exhibit antimalarial and antioxidant activities, with some demonstrating both.

Table 2. Antioxidant Activity Using ABTS Assay

Tested fraction	Conc (µg/mL)						
	10	20	40	60	80	100	IC_{50} ±SE
	% Inhibition						
L-ascorbic acid	24.2	37.4	54.9	66.7	78.5	94.3	29.47±0.17
EA	16.1	29.4	37.2	51.6	66.7	78.1	48.25±0.23
Pet. ether	18.5	25.1	30.7	36.2	45.1	64.0	82.22±0.46
DCM	15.3	23.7	29.0	34.3	43.8	59.4	93.95±0.41
n-Butanol	7.4	16.8	24.5	31.4	38.1	46.2	126.90±0.53
Total	11.8	24.2	32.6	42.9	57.2	65.6	65.51±0.32

31-Norcyclolaudenone and 31-Norcyclolaudenol, are cycloartane triterpenoids. Triterpenes, as cited in [28] and [29], have antimalarial activity. They likely operate through two primary mechanisms. Firstly, their functional groups facilitate hydrogen bonding with the parasite's target sites: 31-Norcyclolaudenone's ketone group acts as a hydrogen bond acceptor, while 31-Norcyclolaudenol's hydroxyl group serves as a hydrogen bond donor. This aligns with observations that triterpenes possessing hydrogen bonding sites often exhibit antimalarial activity [30]. Secondly, these compounds may disrupt the malaria parasite's haem detoxification process: During hemoglobin digestion by *Plasmodium*, toxic-free haem is produced and neutralized by converting it into hemozoin. Many antimalarial drugs interfere with this process, causing toxic haem accumulation and parasite death. 31-Norcyclolaudenone and 31-Norcyclolaudenol might similarly bind to haem, preventing detoxification and causing toxic haem buildup [31].

***β*-Sitosterol, stigmasterol, and *β*-sitosterol-3-*O*-*β*-D-glucoside** are phytosterols, and phytosterol glucosides are reported to possess antimalarial activity [32]. They may act through two main mechanisms:1- Cholesterol Competition: Phytosterols compete with host cholesterol, hindering *Plasmodium*'s ability to synthesize essential sterol components, thus inhibiting its growth and replication [33].2- Immune Response Modulation: Phytosterols enhance the host's immune response by stimulating immune cells and cytokines, making it harder for the parasite to survive and proliferate [34].
These compounds also exhibit antioxidant activities by:1-Scavenging Free Radicals and directly neutralizing them to reduce oxidative stress.2-Boosting Antioxidant Enzymes by increasing their activity like superoxide dismutase (SOD) and catalase [35].3-Preventing Lipid Peroxidation and stabilizing cell membranes to prevent lipid damage.4-Reducing Inflammation which is linked to oxidative stress [36].

Indole-3-carboxaldehyde is a potent antioxidant due to its phenolic hydroxyl group, which scavenges free radicals like DPPH and ABTS by donating hydrogen atoms to form stable phenoxyl radicals [37]. Derivatives, such as Schiff bases complexed with metals like copper and cobalt, enhance this antioxidant activity through redox cycling, effectively neutralizing reactive oxygen species and providing increased protection against oxidative stress [38].

Methyl 3,4-dihydroxybenzoate, belonging to the class of compounds known as benzoates (specifically a methyl ester derivative of protocatechuic acid), like other phenolic compounds, can neutralize free radicals by donating hydrogen atoms or electrons, protecting cells and tissues from oxidative damage [39]. It may also boost the activity of key antioxidant enzymes like superoxide dismutase (SOD) and catalase, reinforcing the body's defense against oxidative stress [40].

Adenosine, a nucleoside consisting of an adenine molecule attached to a ribose sugar, also possesses both antioxidant [41] and antimalarial activities [42]. Adenosine exhibits direct antioxidant effects by scavenging free radicals and reactive oxygen species (ROS), thereby reducing oxidative stress and protecting cells from damage. Additionally, by binding to A_{2A} and A_{2B} adenosine receptors, adenosine modulates inflammatory responses, which are closely associated with oxidative stress [43]. Adenosine analogs disrupt malaria parasite nucleic acid metabolism, essential for its survival in human red blood cells. Although adenosine isn't clinically used due to rapid metabolism and systemic effects, its analogs show promise for new antimalarial therapies [44].

Correlation between the antioxidant activity of the extract and its antimalarial potential:

- In malaria, the *Plasmodium* parasite induces oxidative stress in red blood cells by producing reactive oxygen species (ROS) as part of its metabolic activities, causing damage to both the parasite and the host cells. Supplementing with antioxidants from various sources can help

counteract the oxidative damage caused by the parasite during infection, thereby boosting the overall antioxidant capacity and reducing cellular harm in the host, potentially complementing antimalarial therapies [45].

- As previously discussed in this study, certain compounds isolated from *M. paradisaca* exhibit dual activity as both antimalarial and antioxidant agents. These compounds are: *β*-Sitosterol, stigmasterol, *β*-sitosterol-3-*O*-*β*-D-glucoside, and adenosine.
- The combined antioxidant and antimalarial effects of phytochemicals in the plant extracts can have synergistic effects, enhancing the overall therapeutic potential of the extracts. The antioxidant activity helps protect host cells and tissues, while the antimalarial activity directly targets the parasite.
- Antioxidants play a crucial role in supporting the host's immune system enhancing the body's ability to combat infections and maintain overall health [46].

CONCLUSION

To sum up, *M. paradisiaca* rhizome has been deemed a promising and cheap source of bioactive compounds. More importantly, this study has provided substantial evidence that different extracts of *M. paradisiaca* rhizome possess significant biological activities that could be used for therapeutic applications as anti-malarial and antioxidant. Overall, the potential of these extracts emphasizes the value of natural products in drug discovery and development. Future studies should aim to isolate and elucidate the specific active compounds responsible for these effects, evaluate their mechanisms of action, and assess their efficacy and safety *in vivo*. This could lead to the development of novel therapeutic agents that utilize the wasted parts of *Musa paradisiaca.*

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Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Da-Yong, L.; Ting-Ren, L. Herbal medicine in new era. *Hosp. Palliat. Med*. **2019**, *3* (4), 125-130.
2. Liu, A. Z.; Kress, W. J.; Li, D. Z. Phylogenetic analyses of the banana family (Musaceae) based on nuclear ribosomal (ITS) and chloroplast (trnL-F) evidence. *Taxon* **2010**, *59* (1), 20-28.
3. Vilhena, R. D. O.; Marson, B. M.; Budel, J. M.; Amano, E.; Messias-Reason, I. J. D. T.; Pontarolo, R. Morpho-anatomy of the inflorescence of Musa paradisiaca. *Rev. Bras. Farmacogn*. **2019**, *29*, 147-151.
4. Galani, V. *Musa paradisiaca* Linn.-A Comprehensive Review. *Sch. Int. J. Tradit. Complement. Med.* **2019**, 45-56
5. Adeolu, A. T.; Enesi, D. O. Assessment of proximate, mineral, vitamin, and phytochemical compositions of plantain (Musa paradisiaca) bract–an agricultural waste. *Int. Res. J. Plant Sci.* **2013**, *4* (7), 192-197.
6. Kibria, A. A.; Rahman, M. M.; Kar, A. Extraction and evaluation of phytochemicals from banana peels and banana plants. *Malays. J. Halal Res*. **2019**, *2* (1), 22-26.
7. Shruthi, D. Medicinal uses of banana (Musa paradisiaca). *Drug Invent. Today* **2019**, *12*(1)
8. Kumar, K. S.; Bhowmik, D.; Duraivel, S.; Umadevi, M. Traditional and medicinal uses of banana. *J. Pharmacogn. Phytochem.* **2012**, *1* (3), 51-63.
9. De Langhe, E.; Vrydaghs, L.; de Maret, P.; Perrier, X.; Denham, T. Why Bananas Matter: An introduction to the history of banana domestication. *Ethnobot. Res. Appl.* **2009**, *7*(1), 165-177.
10. Talapko, J.; Škrlec, I.; Alebić, T.; Jukić, M.; Včev, A. Malaria: the past and the present. *Microorganisms* **2019**, *7* (6), 179.
11. Haldar, K.; Mohandas, N. Malaria, Erythrocytic Infection, and Anemia. In *ASH Education Program Book*; American Society of Hematology, Washington, DC, USA, **2009**, pp. 87-93.
12. Lal, A. A.; Rajvanshi, H.; Jayswar, H.; Das, A.; Bharti, P. K. Malaria elimination: Using past and present experience to make malaria-free India by 2030. *J. Vector Borne Dis.* **2019**, *56*(1), 60-65.
13. World Health Organization. (2022). World malaria report. Retrieved from https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022. Accessed on 3 May 2024.
14. Neha, K.; Haider, M. R.; Pathak, A.; Yar, M. S. Medicinal prospects of antioxidants: A review. *Eur. J. Med. Chem.* **2019**, *178*, 687-704.

15. Zehiroglu, C.; Ozturk Sarikaya, S. B. The importance of antioxidants and place in today's scientific and technological studies. *J. Food Sci. Technol.* **2019**, *56*, 4757-4774.
16. Genwali, G. R.; Acharya, P. P.; Rajbhandari, M. Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal J. Sci. Technol.* **2013**, *14*(1), 95-102.
17. D'Alessandro, S.; Silvestrini, F.; Dechering, K.; Corbett, Y.; Parapini, S.; Timmerman, M.; Taramelli, D. A *Plasmodium falciparum* screening assay for anti-gametocyte drugs based on parasite lactate dehydrogenase detection. *J. Antimicrob. Chemother.* **2013**, *68*(9), 2048-2058
18. Valdés, A. F. C.; Martínez, J. M.; Lizama, R. S.; Gaitén, Y. G.; Rodríguez, D. A.; Payrol, J. A. In vitro antimalarial activity and cytotoxicity of some selected Cuban medicinal plants. *Rev. Inst. Med. Trop. Sao Paulo* **2010**, *52*, 197-201
19. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*(9-10), 1231-1237.
20. Knapp, F. F.; Nicholas, H. J. The isolation of 31-norcyclolaudenone from Musa sapientum. Steroids **1970**, *16*, 329-351.
21. Pierre, L. L.; Moses, M. N. Isolation and characterisation of stigmasterol and β-sitosterol from Odontonema strictum (Acanthaceae). *J. Innov. Pharm. Biol. Sci*. **2015**, *2*(1), 88-95.
22. Ajijolakewu, K. A.; Ayoola, A. S.; Agbabiaka, T. O.; Zakariyah, F. R.; Ahmed, N. R.; Oyedele, O. J.; Sani, A. A review of the ethnomedicinal, antimicrobial, and phytochemical properties of *Musa paradisiaca* (plantain). *Bull. Natl. Res. Cent.* **2021**, *45* (1), 86.
23. Akihisa, T.; Kimura, Y.; Tamura, T. Cycloartane triterpenes from the fruit peel of *Musa sapientum*. *Phytochemistry* **1998**, *47* (6), 1107-1110.
24. Lakshmi, V.; Agarwal, S. K.; Ansari, J. A.; Mahdi, A. A.; Srivastava, A. K. Antidiabetic potential of *Musa paradisiaca* in Streptozotocin-induced diabetic rats. J. *Phytopharmaco*l. **2014**, *3* (2), 77-81.
25. Martínez-Luis, S.; Gómez, J.F.; Spadafora, C.; Guzmán, H.M.; Gutiérrez, M. Antitrypanosomal alkaloids from the marine bacterium Bacillus pumilus. Molecules 2012, 17 (9), 11146–11155.
26. Pouramini, Z.; Moradi, A. Structural and orthoselectivity study of 2-hydroxybenzaldehyde using spectroscopic analysis. *Arab. J. Chem*. **2012**, *5* (1), 99-102.
27. Seo, D. J.; Kim, K. Y.; Park, R. D.; Kim, D. H.; Han, Y. S.; Kim, T. H.; Jung, W. J. Nematicidal activity of 3,4-dihydroxybenzoic acid purified from *Terminalia nigrovenulosa* bark against *Meloidogyne incognita*. *Microb. Pathog*. **2013**, *59*, 52-59.
28. Ciuffreda, P.; Casati, S.; Manzocchi, A. Complete 1H and 13C NMR Spectral Assignment of α- and β-Adenosine, 2′-Deoxyadenosine and Their Acetate Derivatives. *Magn. Reson. Chem*. **2007**, *45* (9), 781-784.
29. Ramalhete, C.; Lopes, D.; Mulhovo, S.; Molnár, J.; Rosário, V. E.; Ferreira, M. J. U. New antimalarials with a triterpenic scaffold from *Momordica balsamina*. Bioorg. *Med. Chem*. **2010**, *18* (14), 5254-5260.
30. Ramalhete, C.; da Cruz, F. P.; Mulhovo, S.; Sousa, I. J.; Fernandes, M. X.; Prudêncio, M.; Ferreira, M. J. U. Dual-stage triterpenoids from an African medicinal plant targeting the malaria parasite. *Bioorg. Med. Chem*. **2014**, *22* (15), 3887-3890.
31. Nasomjai, P.; Arpha, K.; Sodngam, S.; Brandt, S. D. Potential antimalarial derivatives from astraodorol. *Arch. Pharmacal Res*. **2014**, *37*, 1538-1545.
32. da Silva, G. N.; Maria, N. R.; Schuck, D. C.; Cruz, L. N.; de Moraes, M. S.; Nakabashi, M.; Gnoatto, S. C. Two series of new semisynthetic triterpene derivatives: differences in anti-malarial activity, cytotoxicity and mechanism of action. *Malar. J.* **2013**, *12*, 1-7.
33. Irungu, B. N.; Adipo, N.; Orwa, J. A.; Kimani, F.; Heydenreich, M.; Midiwo, J. O.; Erdélyi, M. Antiplasmodial and cytotoxic activities of the constituents of *Turraea robusta* and *Turraea nilotica*. *J. Ethnopharmacol***. 2015**, *174*, 419-425.
34. Maier, A. G.; van Ooij, C. The role of cholesterol in invasion and growth of malaria parasites. *Front. Cell. Infect. Microbiol*. **2022**, *12*, 984049. https://doi.org/10.3389/fcimb.2022.984049
35. Bouic, P. J. The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. *Curr. Opin. Clin. Nutr. Metab. Care* **2001**, *4* (6), 471-475.
36. Yoshida, Y.; Niki, E. Antioxidant effects of phytosterol and its components. *J. Nutr. Sci. Vitaminol*. **2003**, *49* (4), 277-280.
37. Wang, T.; Hicks, K. B.; Moreau, R. Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates. *J. Am. Oil Chem. Soc*. **2002,** *79* (12), 1201-1206
38. Manimalathi, S.; Priya, J.; Madheswari, D. Exploration on Synthesis, Characterization and Biological Activities of Transition Metal Complexes Comprising Hydroxybenzylideneaminocyclohexylimino-methyl-4, 6-dibromophenol Schiff Base Ligand. *Arab. J. Sci. Eng.* **2024**, 1-21.
39. Soroceanu, A.; Bargan, A. Advanced and biomedical applications of Schiff-base ligands and

their metal complexes: A review. *Crystals* **2022**, *12* (10), 1436
40. Zhang, S.; Gai, Z.; Gui, T.; Chen, J.; Chen, Q.; Li, Y. Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: old wine in a new bottle. *Evid. Based Complement. Alternat. Med.* **2021**, *20* (1), 6139308.
41. El-Sonbaty, Y. A.; Suddek, G. M.; Megahed, N.; Gameil, N. M. Protocatechuic acid exhibits hepatoprotective, vasculoprotective, antioxidant and insulin-like effects in dexamethasone-induced insulin-resistant rats. *Biochimie* **2019**, *167*, 119-134.
42. Maggirwar, S. B.; Dhanraj, D. N.; Somani, S. M.; Ramkumar, V. Adenosine acts as an endogenous activator of the cellular antioxidant defense system. *Biochem. Biophys. Res. Commun*. **1994**, *201* (2), 508-515.
43. Rodenko, B.; van der Burg, A. M.; Wanner, M. J.; Kaiser, M.; Brun, R.; Gould, M.; Koomen, G. J. 2, N6-disubstituted adenosine analogs with antitrypanosomal and antimalarial activities. *Antimicrob. Agents Chemother.* **2007**, *51* (11), 3796-3802
44. Ramkumar, V.; Hallam, D. M.; Nie, Z. Adenosine, oxidative stress and cytoprotection. Jpn. *J. Pharmacol.* **2001,** *86* (3), 265-274.
45. Coomber, D. W.; O'Sullivan, W. J.; Gero, A. M. Adenosine analogues as antimetabolites against *Plasmodium falciparum* malaria. *Int. J. Parasitol*. **1994**, *24* (3), 357-365.
46. Gomes, A. R. Q.; Cunha, N.; Varela, E. L. P.; Brígido, H. P. C.; Vale, V. V.; Dolabela, M. F.; Percário, S. Oxidative stress in malaria: potential benefits of antioxidant therapy. *Int. J. Mol. Sci.* **2022**, *23* (11), 5949.
47. Puertollano, M. A.; Puertollano, E.; Alvarez de Cienfuegos, G.; de Pablo, M. A. Dietary antioxidants: immunity and host defense. *Curr. Top. Med. Chem.* **2011**, *11* (14), 1752-1766