Trop J Nat Prod Res, November 2024; 8(11): 9097 - 9103 **ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

# Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

# *β*- Hematin and Nitric Oxide Inhibitory Activities of Ten Nigerian Medicinal Plants

Gbemisola M. Ojobaro, Abraham O. Nkumah, Saka Y. Alabi, Thomas Abu. Zainab A. Molik, Omonike O. Ogbole*.

*Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.*

ARTICLE INFO

*Article history:*
Received 15 February 2024
Revised 16 February 2024
Accepted 11 November 2024
Published online 01 December 2024



ABSTRACT

Ten Nigerian medicinal plants, known for their antimalarial properties yet with unknown mechanisms of action, were selected to investigate whether their effects result from inhibiting *β*-Hematin (*β*-H) formation, a recognized antimalarial mode of action. Previous research has shown that nitric oxide (NO) activity protects *Plasmodium falciparum* in vitro. However, it has been hypothesized that excessive NO production might contribute to the pathogenesis of cerebral malaria. Selected plant parts were extracted in methanol and concentrated *in vacuo*. *β*-Hematin and nitric oxide (NO) inhibitory assays were carried out on the extracts, and chloroquine and ascorbic acid were used as respective standards. The most active plant extract was fractionated into n-hexane, dichloromethane, ethyl acetate, and butanol. The n-hexane fraction of *Rauvolfia vomitoria* leaves underwent gas chromatography-mass spectrometry (GC-MS) analysis to identify its suggested compounds or components present in the fraction.The selected plants exhibited varying levels of *β*-H and NO inhibitory actions, with *Rauvolfia vomitoria* showing the highest *β*-Hematin inhibitory activity ($IC_{50}$ = 0.22 ± 0.62 µg/mL) compared to chloroquine ($IC_{50}$ = 2.14 ± 0.36 µg/mL). GC-MS analysis of the n-hexane fraction of *Rauvolfia vomitoria* identified 12 major compounds, with Neophytadiene (43.30%) being the most abundant. *Chromolaena odorata* leaves demonstrated the highest NO inhibitory activity ($IC_{50}$ = 19.98 ± 0.30 µg/mL) compared to ascorbic acid ($IC_{50}$ = 0.15 ± 0.12 µg/mL). The extract of *Rauvolfia vomitoria* significantly inhibited *β*-Hematin formation more effectively than chloroquine. This study confirms the traditional claim of the selected plant scientifically.

***Keywords:*** Antimalarial, Medicinal plants, *β*-hematin, Nitric oxide, Gas Chromatography-Mass Spectrometry.

## Introduction

Malaria remains a significant global health burden, particularly in tropical regions where traditional medicinal plants are often used as antimalarial remedies. The ongoing development of *Plasmodium falciparum* antimalarial drug resistance is a serious threat to malaria control strategies. In this situation, medicinal plants provide complementary therapies with significant promise since chemotherapeutic medicines are in demand and the search for novel antimalarial medications is a top priority [1]. It is crucial to determine the effectiveness, safety, and mode of action of these traditional medicinal herbs used to treat malaria and generate novel antimalarial medications. The remark
able success of artemisinin and its semi-synthetic derivatives as potent plant-derived antimalarials, along with the historical importance of quinine from Cinchona species (and the development of synthetic analogs such as chloroquine and mefloquine), has driven continuous efforts to discover new antimalarials derived from natural products. This ongoing search is motivated by the need for novel treatments due to the rise of drug-resistant strains of malaria [2].

*Corresponding author. E-mail: nikeoa@yahoo.com
Tel: +2348056434577

**Citation:** Ojobaro MG, Nkumah AO, Alabi SY, Abu T, Molik ZA, Ogbole OO. *β* -Hematin and Nitric Oxide Inhibitory Activities of Ten Nigerian Medicinal Plants. Trop J Nat Prod Res. 2024; 8(11): 9097 – 9103
https://doi.org/10.26538/tjnpr/v8i11.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Many medicinal plants in Africa, have been established to have antimalaria properties in literature but their mechanism of action has not been detailed [3]. There have been pharmacological evidences that the ten (10) selected Nigeria medicinal plants used in this study *Tetrapleura tetraptera* [4], *Newboudia laevis* [5], *Parkia biglobosa* [6], *Ficus thonnigii* [6], *Piper guineense* [7], *Chrysophyllum albidum* [8], *Rauvolfia vomitoria* [9], *Carica papaya* [10], *Chromolaena odorata* [11], *Garcinia kola* [12], possess antimalarial activities but their mechanisms of action have not been evaluated and established.

Hemozoin, an insoluble dark brown crystalline solid known as malaria pigment, is a crystalline by-product of hemoglobin breakdown. It aids in the diagnosis of malaria parasites and can be found in the patient's blood smear, the synthetic variant of hemozoin has a crystal structure known as *β*-hematin [13]. From the perspective of a drug target, this pathway has been validated for several antimalarial molecules including the 4-aminoquinolines (quinine, mefloquine, amodiaquine, and chloroquine), and this is therefore considered a suitable target for new molecules in drug discovery [14]. The *β*-hematin inhibitory assay is regarded as a reliable assay for determining the mechanism of action of antimalarial drugs that utilize the pathway [15]. The inhibition of *β*-hematin formation by an extract is an indication of the possible mechanism of action as an antimalarial agent [16].

Nitric oxide (NO), while essential for various physiological processes, can become harmful when it combines with reactive oxygen species (ROS). This interaction contributes to oxidative stress, which can lead to cellular damage due to the non-specific nature of the oxidative process. When NO reacts with superoxide radicals ($O_2^-$), it forms peroxynitrite ($ONOO^-$), a potent oxidant that can further damage cellular components, such as lipids, proteins, and DNA. This compound can overwhelm the body's antioxidant defence systems, resulting in increased oxidative stress and promoting inflammatory or degenerative conditions [17]. Nitric oxide inhibitors compete with oxygen, which reduces the synthesis of nitrite ions [18]. From the standpoint of a drug target, the nitric oxide inhibitory assay is one of the techniques that can

be used to determine antioxidant activity *in vitro*. This method has been validated for gallic and ascorbic acid, so this is thought to be a suitable target for antioxidant molecules in drug discovery [19]. During acute malaria, there is a substantial correlation between increased NO generation and reduced hemoglobin (Hb) levels. Therefore, increased NO generation may contribute to the development of malaria anemia (MA). Because NO may decrease erythropoiesis [20] and induce apoptosis [21] in hematopoietic progenitors, overly high NO levels during acute malaria may be a factor in this process inhibition. However, the suppression of NO generation might increase Hb levels, which impedes the development of malaria.

In recent years, the combined analytical method known as gas chromatography-mass spectroscopy (GC-MS) has been utilized to determine and identify the chemicals contained in plant samples as well as non-plant species [22]. GC-MS plays an essential role in the phytochemical analysis studies of medicinal plants containing biologically active components [22]. This study presents a novel exploration into the antimalarial properties of ten established medicinal plants through the use of *β*-hematin inhibition assay. By integrating an investigation of their nitric oxide inhibitory activity and identifying active compounds via GC-MS analysis, this research provides new insights into the mechanisms of action of these plants. This comprehensive approach not only suggests the bioactive components responsible for their antimalarial effects but also contributes valuable data to the field of ethnopharmacology, potentially leading to the development of new antimalarial therapies.

## Materials and Methods

*Plant collection and authentication*

The dried fruits of *Tetrapleura tetraptera*, leaves of *Newboudia laevis*, stem bark of *Parkia biglobosa*, leaves of *Ficus thonnigii*, stem bark of *Chrysophyllum albidum*, leaves of *Rauwolfia vomitoria* and nuts of *Garcinia kola* were collected at RW 34+ 25 MF, Ago-Iwoye, Ijebu Ode 120103, Ogun State, Nigeria while leaves of *Carica papaya*, stem bark of *Chromolaena odorata* and leaves of *Piper guineense* were collected at CW56+P9, Olorunda Akobo Ibadan, Oyo State, Nigeria. The Forest Herbarium Ibadan (FHI), Oyo State, Nigeria, where voucher specimens were placed, was where plants were identified and validated and FHI numbers were allocated.

*Preparation of plant materials*

Plant samples were prepared and allowed to air dry for two weeks in the shade. They were pulverized and macerated into absolute methanol for 72 hours at room temperature and concentrated using a Rotary Evaporator (Pengertian Fungsi Bagian Prinsip Ke vogue co) and extracts were thereafter stored in -4°C refrigerator until further use. The most potent plant extract was further partitioned into n-hexane, dichloromethane (DCM), ethyl-acetate, butanol, and aqueous-methanol

*In vitro* assays

*β - hematin inhibitory assay*

The ability of the crude extracts and fractions to inhibit the formation of *β* - hematin *in vitro* was determined using the method of Vargas et al., [23] and modified by Wande and Babatunde [24]. The crude extracts and fractions were prepared in 10-fold dilution in six graded concentrations (1000,100,10,1.0,0.1 and 0.01, µg/mL) and chloroquine phosphate (Sigma Aldrich) was used as standard.

*Nitric oxide inhibitory assay*

The capacity of the crude extracts to inhibit the formation of the stable nitric oxide free radical was determined, using a modification of the Griess reaction method of Shakya *et al*., [25] mixture of 10 µL of 10 mM sodium nitroprusside and 100 µL of various concentrations (31.25–1000 µg/mL) of the extract and fractions were prepared in serial dilutions using phosphate buffer (pH 7.4). The 96-well plate containing these mixtures was incubated for 2.5 hours at 25°C. Following incubation, 100 µL of Griess reagent (containing 2% *O*-phosphoric acid, 1% sulphanilamide, and 1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to the mixture and absorbance was read at 564nm using (Molecular Devices SpectraMax M5 Multi-Mode Microplate Reader). Ascorbic acid was used as the standard.

*Gas chromatography-mass spectrometry (GC-MS) analysis*

Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II, a GC-MS device from Thermo Scientific Co., was used to conduct the phytochemical analysis of the methanolic extract. The GC-MS system underwent the following experimental conditions: TR 5-MS capillary standard non-polar column, 30Mts in length, 0.25 mm ID, and 0.25 m Film Thickness. Mobile phase flow was set at 1.0 ml/min (carrier gas: He). In the gas chromatography section, the injection volume was 1 l, and the temperature programmed (oven temperature) was 40°C rising to 250°C at 5°C/min. Using the Wiley Spectral library search tool, the findings of samples that had been thoroughly run at a range of 50-650 *m/z* were compared

*Compounds' identification*

Utilizing the database of the National Institute of Standards and Technology, components were identified based on their retention indices, and mass spectra were interpreted (NIST). The *Rauvolfia vomitoria* n-hexane fraction acquired spectra of the unknown components were compared with the standard mass spectra of the known components kept in the NIST collection.

*Statistic evaluation*

The means and standard error of the means (SEM) were used to express the results and analysed using Prism Graphpad® (5.0).

## Results and Discussion

This finding suggests that novel antimalarial leads may undoubtedly come from sources of tropical plants since natural products have played a prominent role in the identification of lead molecules for the development of medications to treat human illnesses [26]. Numerous novel medications have been created and put on the market as a result of the effective exploitation of nature as a source of bioactive compounds [27]. Malaria remains the most devastating parasitic disease in many tropical and subtropical regions, with its impact steadily increasing. The rise of resistance in *Plasmodium falciparum*, the parasite responsible for the most severe cases of malaria, to commonly used antimalarial drugs has intensified the challenge of controlling the disease. This growing resistance underscores the urgent need for new, effective, and affordable antimalarial treatments that have clearly defined modes of action. Innovative therapies must address drug resistance, improve accessibility, and be economically viable to combat the increasing threat of malaria effectively, particularly in regions where the burden is highest [28].

The *β*-hematin inhibitory assay is considered a reliable assay for determining the mechanism of action of antimalarial drugs [15]. This test is based on the ability of the ten plant extracts (Table 1) to prevent the production of hemozoin in a manner analogous to that of the common medication chloroquine. As demonstrated in Table 2, all of the plants' methanol extracts evaluated for this investigation showed varying degrees of hemozoin production inhibition in comparison to the standard drug chloroquine ($IC_{50}$ = 2.14 ± 0.36 µg/mL), the methanol extract of the leaves of *R. vomitoria*, the fruit of *T. tetraptera*, the bark of *P. biglobosa*, and the leaves of *P. guineense* had more pronounced *β*-hematin inhibitory activity ($IC_{50}$ = 0.22 ± 0.62 µg/mL, 0.36 ± 0.51 µg/mL, 1.66 ± 0.42 µg/mL, 1.68 ± 0.52 µg/mL) respectively. The phytochemicals present in these plants may be responsible for the *β*-hematin inhibition. These results suggest that the methanol extracts of these plant parts (*R. vomitoria* leaves, *T. teraptera* fruits, *P. biglobosa* bark, and *P. guineense* leaves) use a pathway similar to that of chloroquine for their mechanism of action and may be further explored for the discovery of bioactive components responsible for *β*-hematin inhibitory activity.

*In vitro*, nitric oxide inhibitory assay is based on the ability of the extracts to inhibit the production of nitric oxide free radicals using

ascorbic acid as a standard drug[18]. All of the crude extracts examined for this investigation showed varying degrees of NO inhibition. With the use of GraphPad prism, the plants' 50% inhibitory concentrations ($IC_{50}$), which are shown in Table 2 as a function of their nitric oxide inhibitory activities, were determined.

According to the findings, among all the plant extracts, the methanol extract of *C. odorata* leaves showed the most significant nitric oxide inhibitory action ($IC_{50}$ = 19.98 ± 0.30 µg/mL), comparable to ascorbic acid ($IC_{50}$ = 0.15 ± 0.12 µg/mL). The ability of *C. odorata* leaves to suppress nitric oxide production can be ascribed to any specific component or group of compounds. A prior investigation was reported by [24]. The phenolic and flavonoid content of *C. odorata* leaves has been proven to be high in anti-inflammatory and antioxidant properties, for example, they are advantageous to humans and serve as key preventative measures against disease processes caused by free radicals [29, 30].

**Table 1:** Identification and authentication of plants at Forest Herbarium, Ibadan

| Voucher Specimen | Family | Voucher Specimen Number |
|---|---|---|
| *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. | Fabaceae | FHI 112865 |
| *Newboudia laevis (*P.Beauv.) Seem. ex Bureau. | Bignoniaceae | FHI 112869 |
| *Parkia biglobosa* (Jacq.) R. B R.ex G.Don | Fabaceae | FHI 112864 |
| *Ficus thonnigii* Blume. | Moraceae | FHI 112885 |
| *Piper guineense* Schumach. | Piperaceae | FHI 112887 |
| *Chrysophyllum albidum* G. Don. | Sapotaceae | FHI 112862 |
| *Rauvolfia vomitoria* Afzel. | Apocynaceae | FHI 112866 |
| *Carica papaya* L. | Caricaceae | FHI 112863 |
| *Chromolaena odorata* (L.) R. M. King & H. Rob. | Asteraceae | FHI 112861 |
| *Garcina kola* Heckel. | Clusiaceae | FHI 112886 |

The methanol extract of leaves of *R. vomitoria* was observed to be the most active in inhibiting *β*- hematin and also have appreciable NO inhibition with ($IC_{50}$= 27.98 ± 0.28 µg/mL) (Table 2). The extract was thereafter partitioned into n-hexane, dichloromethane, ethyl acetate, butanol, and aqueous fractions (according to their order of polarity) and was further screened for *β*- hematin inhibitory assay. The n-hexane fraction had the most active *β*-hematin inhibitory activity ($IC_{50}$= 0.06 ±0.04 µg/mL) compared to chloroquine ($IC_{50}$ = 2.14 ±0.36 µg/mL) as shown in Figure 1. However, the connotation between high NO production and reduced Haemoglobin (Hb) levels during acute malaria [20]. Thereby the activities observed in the *R. vomitoria* methanolic extract in inhibiting *β*- hematin could be in association with its appreciable NO inhibition.

The GC-MS result (Table 3) (Figure 2 and 3) revealed twelve major compounds in the n-hexane fraction of *R. vomitoria* leaves. The n-hexane fraction of *R. vomitoria* leaves contains a complex blend of pyridines and derivatives, alkaloids, terpenoids, alcohols, fatty acid esters, and other phytochemicals. However, the major compounds identified were Neophytadiene (43.30 %), Benzeneethanamine,4-methoxy-alpha-methyl (14.60 %), Glutaraldehyde (10.98 %), 5-Nitro-3-cyano-2(1H)-pyridone (8.37 %), 2-Butanamine, 3,3-dimethyl- (8.12 %), Cyclobutanol (3.10 %). The biological activities reported for this plant may be due to the presence of these phytochemicals' constituents. For example; the most abundant compound, Neophytadiene (43.30 %) is a diterpene ( 3-methylidenehexadec-1-ene), that has been reported for cytotoxicity and antioxidant activities in essential oils of *Parquetina nigrescens* (afz.) [31] as well as *in vivo* anti-inflammatory properties in *Turbinaria ornata* [32], for the treatment of rheumatoid arthritis and antimicrobial activity [33]. Glutaraldehyde (10.98 %), is another major compound present and is known to be naturally occurring in several medicinal plants. It has been reported for its potent antifungal [34] and cytotoxicity activities [35]. 5-Nitro-3-cyano-2(1H)-pyridone (8.37 %), is a pyridine and quinolone derivative. There are several pyridine derivatives in nature, and because of their diverse biological properties, such as their ability to serve as antibacterial, anticancer, antioxidant, and insecticidal, therefore, they have drawn the attention of chemists all over the world [36, 37] . Many quinoline derivatives are also biologically active and are used as antimalarial and anti-inflammatory medications. The natural and synthesized form of Cyclobutanol (3.10 %) has been reported for antibacterial and antioxidant activities [38].

**Table 2:** *β* - hematin and Nitric oxide $IC_{50}$ (µg/mL) of the Ten (10) plant extracts compared to Chloroquine and Ascorbic acid.

| Plants | Parts | *β* - hematin | Nitric oxide |
|---|---|---|---|
| | | $IC_{50}$ (µg/mL) | $IC_{50}$ (µg/mL) |
| Chloroquine | Standard drug | 2.14 ± 0.36 | - |
| Ascorbic acid | Standard drug | - | 0.15 ± 0.12 |
| *Rauvolfia vomitoria* | Leaves | 0.22 ± 0.62 | 27.98 ± 0.28 |
| *Tetrapleura tetraptera* | Fruit | 0.36 ± 0.51 | 25.92 ± 0.32 |
| *Parkia biglobosa* | Stem bark | 1.66 ± 0.42 | 30.33 ± 0.34 |
| *Piper guineense* | Leaves | 1.68 ± 0.52 | 97.28 ± 0.33 |
| *Ficus thonnongi* | Leaves | 2.89 ± 0.51 | 24.67 ± 0.34 |
| *Garcinia kola* | Nuts | 3.20 ± 0.41 | 27.49 ± 0.32 |
| *Chromolena odorata* | Leaves | 17.95 ± 0.22 | 19.98 ± 0.30 |
| *Chrysophyllum albidum* | Stem bark | 36.13 ± 0.63 | 62.46 ± 0.29 |
| *Carica papaya* | Leaves | 39.98 ± 0.21 | 1001.23± 0.32 |
| *Newbouldia laevis* | Leaves | 51.78 ± 0.20 | 223.29 ± 0.34 |

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 3:** Compounds identified from n-hexane fraction of *R. vomitoria* from GC-MS

| Peak no | Retention time (min) | Compound name | Molecular formula | Molecular weight (g/mol) | Peak Area % |
|---|---|---|---|---|---|
| **1** | 3.14 | 5-Nitro-3-cyano-2(1H)-pyridone | $C_6H_3N_3O_3$ | 165.11 | 8.37 |
| **2** | 5.27 | Benzeneethanamine, N-methyl- Amphetamine | $C_9H_{13}N$ | 135.20 | 1.43 |
| **3** | 5.63 | Cyclobutanol | $C_4H_7OH$ | 72.11 | 3.10 |
| **4.** | 5.93 | 1-Octanamine, N-methyl- | $C_9H_{21}N$ | 143.26 | 1.93 |
| **5.** | 7.60 | 2-Methoxy-N-methylethylamine | $C_4H_{11}NO$ | 84.14 | 2.08 |
| **6** | 8.87 | Dodecanoic acid, 11-amino-, methylester | $C_{13}H_{27}NO_2$ | 229.36 | 1.37 |
| **7** | 9.67 | Actinobolin | $C_{13}H_{20}N_2O_6$ | 300.31 | 1.75 |
| **8** | 13.91 | Neophytadiene | $C_{20}H_{38}$ | 278.50 | 43.30 |
| **9** | 14.12 | Glutaraldehyde | $C_5H_8O_2$ | 100.11 | 10.98 |
| **10** | 14.28 | Benzeneethanamine, 4-methoxy-. alpha. -methyl | $C_{10}H_{15}NO$ | 165.23 | 14.60 |
| **11** | 14.58 | 2-Butanamine, 3,3-dimethyl- | $C_6H_{15}N$ | 101.19 | 8.12 |
| **12** | 16.16 | 2-Butanamine, 3-methyl- | $C_5H_{13}N$ | 87.16 | 2.97 |

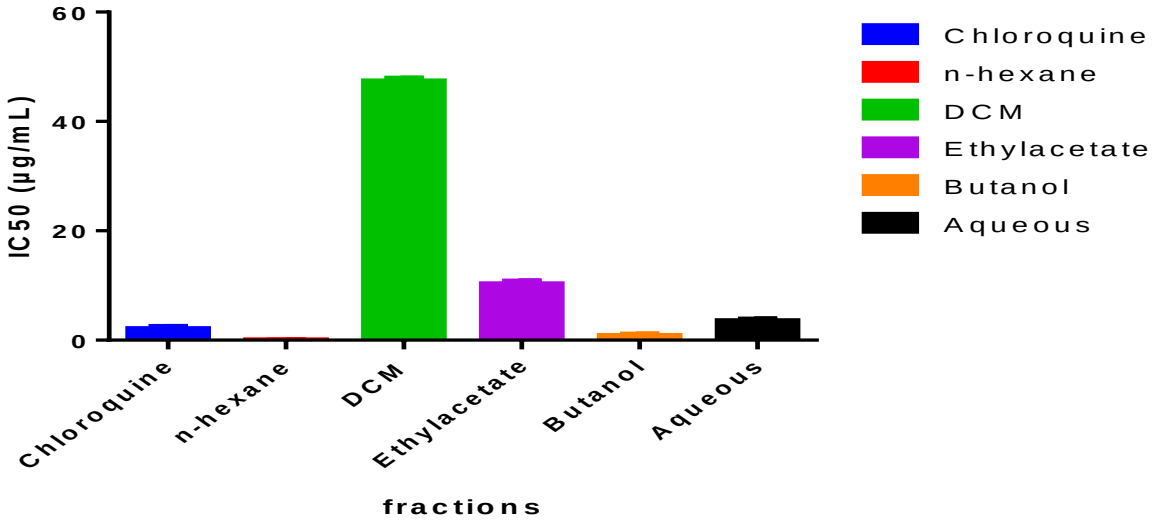


**Figure 1:** *β*-hematin $IC_{50}$ (µg/mL) of *R. vomitoria* leaves partitioned fractions compared to chloroquine

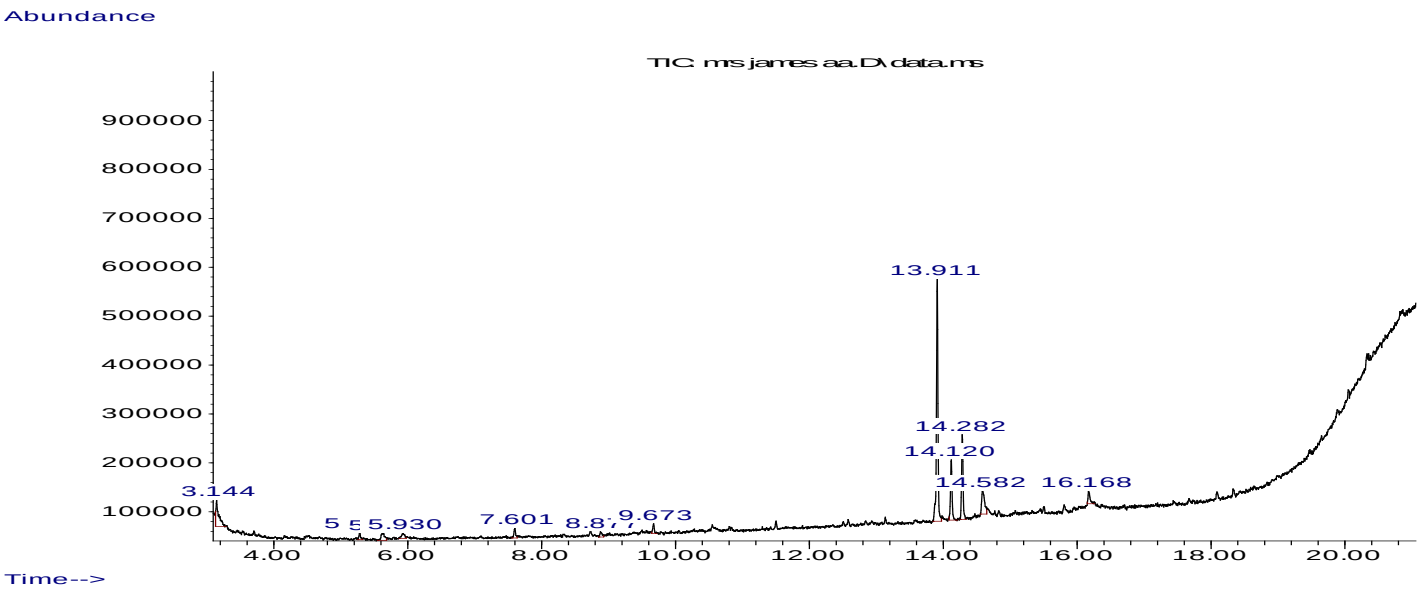


**Figure 2:** Gas Chromatogram of n-Hexane Fraction of *R. vomitoria*

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

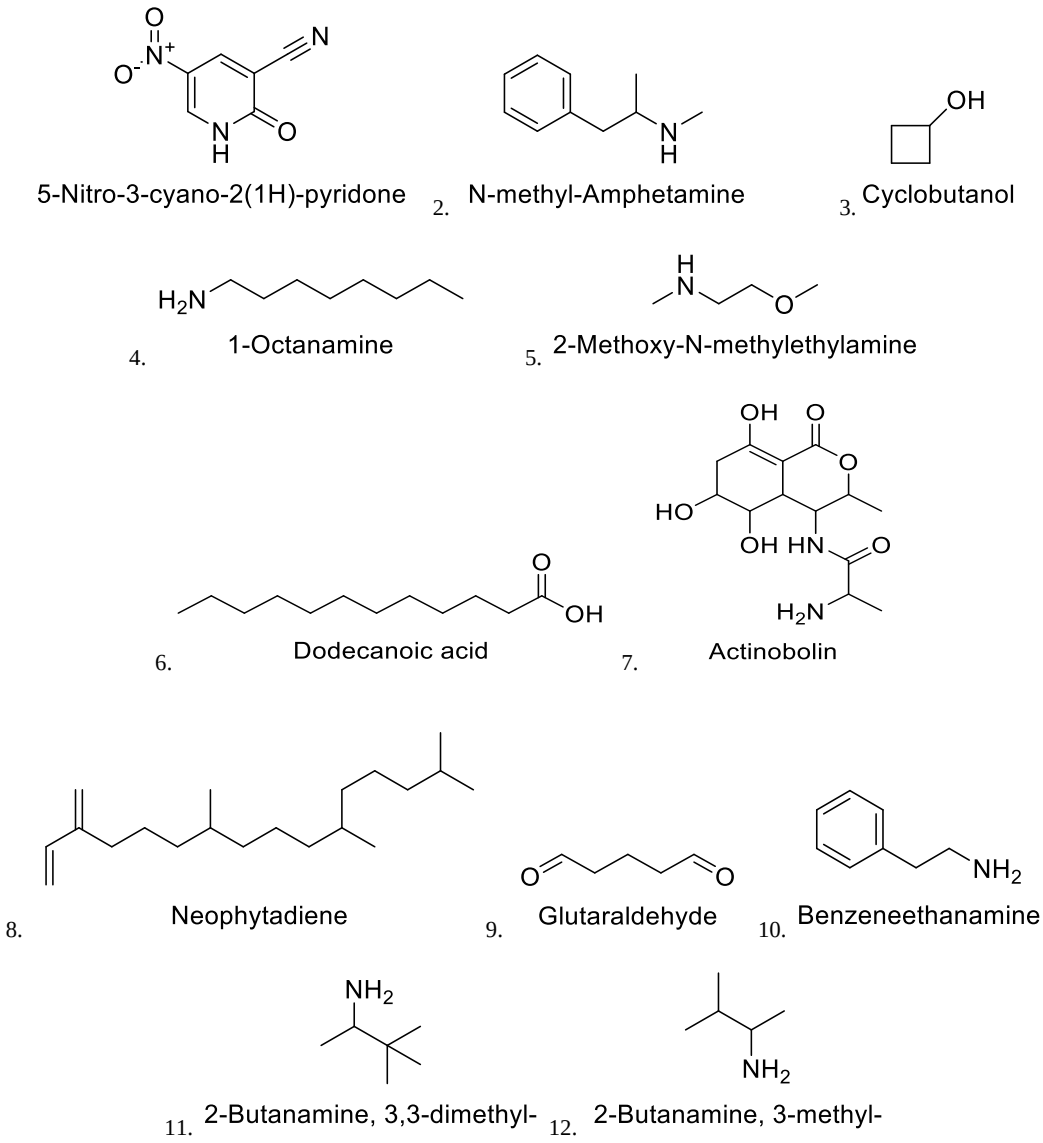


**Figure 3:** Structure of Chemical Compounds Identified from n-Hexane Fraction of *R. vomitoria*

## Conclusion

This investigation was conducted to assess the *β*- hematin and nitric oxide inhibitory activities of ten Nigerian medicinal plants used for treating malaria and determine if their mode of action is via the *β*-hematin pathway. From the results, it is evident that methanol extract from the fruit of *T. tetraptera,* bark of *P. biglobosa*, and leaves of *P. guineense* followed the *β*-hematin inhibitory activity pathway compared to chloroquine, while methanol extract from leaves of *C. odorata* showed the highest nitric oxide inhibitory activity compared to ascorbic acid. However, the methanol leaf extract of *R. vomitoria* was more effective in inhibiting the synthesis of hemozoin as compared to extracts of other plants. Furthermore, the GC-MS results of the n-hexane fraction of *R. vomitoria* leaves show the presence of phytochemical constituents with reported biochemical properties which may justify the nitric oxide and *β*- hematin inhibitory activities of this plant and in turn its antioxidant and antimalarial properties.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## Acknowledgments

The authors sincerely appreciate, the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, and the staff of the laboratory, where this research is carried out. We also appreciate the staff of the Forest Herbarium Ibadan (FHI) for plant identification.

## References

1. Linhares Y, Kaganski A, Agyare C, Kurnaz IA, Neergheen V, Kolodziejczyk B, Kędra M, Wahajuddin M, El-Youssf L, dela Cruz TE, Baran Y. Biodiversity: The Overlooked Source of Human Health. Trends Mol Med. 2023;29(3):173-187.
2. Kingston DGI, Cassera MB. Antimalarial Natural Products. Prog Chem Org Nat Prod. 2022;117:1-106.

3. Cock IE, Selesho MI, van Vuuren SF. A review of the traditional use of southern African medicinal plants for the treatment of malaria. J Ethnopharmacol. 2019; 245:112-176.
4. Odubanjo VO, Ibukun EO, Oboh G, Adefegha SA. Aqueous extracts of two tropical ethnobotanicals, *Tetrapleura tetraptera* and *Quassia undulata* improved spatial and non-spatial working memories in scopolamine-induced amnesic rats: Influence of neuronal cholinergic and antioxidant systems. Biomed Pharmacother. 2018;99:198-204.
5. Salawu MO, Raji NH, Oloyede HO. Antimalarial activity of aqueous stem bark extract of *Newbouldia laevis* in *Plasmodium berghei* infected mice. CPJ. 2020;2020002:26-102.
6. Hassan Y, Umar HZ, Abdulkadir Z, Ahmad HS, Ibrahim FS, Ahmad IN. *In vitro* antiplasmodial potential of crude alkaloids from some west africa antimalarial plants. IJPSR. 2020;11 (07):188-191.
7. Ojo OA. Exploring the potentials of selected bioactive compounds isolated from *Piper guineense* Schumach. and Thonn. leaf toward identification of novel pfDHFR and pfDHODH inhibitors as antimalaria agents. J Appl Pharm. Sci.. 2021;11(4):153-158.
8. Odediran S, Awosode K, Adegoke T, Odebunmi K, Oladunjoye B, Obasanya A. Combinations of *Chrysophyllum albidum* and *Citrus aurantifolia* as antimalarial agents and their effects on orthodox antimalarial drugs in mice. Ann Complement Altern Med. 2020;2(1):100-109.
9. Tlhapi DB, Ramaite IDI, Van Ree T, Anokwuru CP, Orazio TS, Hoppe HC. Isolation, Chemical Profile and Antimalarial Activities of Bioactive Compounds from *Rauvolfia caffra* Sond. Molecules. 2018;24(1):39-49.
10. Teng W-C, Chan W, Suwanarusk R, Ong A, Ho H-K, Russell B, Rénia L, Koh HL. *In vitro* antimalarial evaluations and cytotoxicity investigations of *Carica papaya* leaves and carpaine. Nat Prod Commun. 2019;14(1):33-36.
11. Ejiofor N, Nna PJ. Analysis of the phytoconstituents of *Chromolaena odorata* leaves and its bioactivities against some clinical and plant pathogens. FNAS-JSI. 2022;3(2):1-9.
12. Ujomu TS, Adegoke G, Abiodun O, Gbotosho GO. Antimalarial activity and cytotoxicity profile of the seed extracts of *Garcinia kola*. J phytopharm. 2019;8(6):282-285.
13. Nicoletti M. Three scenarios in insect-borne diseases: Insect-Borne Diseases in the 21st Century. Epub; 2020.99-251. doi: 10.1016/B978-0-12-818706-7.00005-X. PMCID: PMC7442119.
14. Goyal A, Kharkwal H, Piplani M, Singh Y, Murugesan S, Aggarwal A, Kumar P, Chander S. Spotlight on 4-substituted quinolines as potential anti-infective agents: Journey beyond chloroquine. Arch Pharm. (Weinheim). 2023;356(3):220-361.
15. Titilawo Y, Adeniji A, Adeniyi M, Okoh A. Determination of levels of some metal contaminants in the freshwater environments of Osun State, Southwest Nigeria: A risk assessment approach to predict health threat. Chemosphere. 2018; 211:834-843.
16. Olanlokun JO, Adetutu JA, Olorunsogo OO. *In vitro* inhibition of beta-hematin formation and in vivo effects of *Diospyros mespiliformis* and *Mondia whitei* methanol extracts on chloroquine-susceptible *Plasmodium berghei* induced malaria in mice. Interv Med Appl Sci. 2021;11(4):197-206.
17. Radi R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc Natl Acad Sci. U.S.A. 2018;115(23):5839-5848.
18. Arirudran B, Priyadharshini P, Rao UM. Estimation of anti-inflammatory activity as well as apoptotic activity of ethanolic extracts of *Crocus sativus*. Univers J Pharm Res. 2018;3(6):6-11.
19. Ahmed MS, Khan IJ, Aman S, Chauhan S, Kaur N, Shriwastav S. Phytochemical investigations, *in vitro* antioxidant, antimicrobial potential, and *in*-silico computational docking analysis of *Euphorbia milii* Des Moul. J Exp Biol Agric Sci. 2023;11(2):380-389.
20. Gupta M, Kumar S, Kumar R, Kumar A, Verma R, Darokar MP. Inhibition of heme detoxification pathway in malaria parasite by 3-hydroxy-11-keto-*β*-boswellic acid isolated from *Boswellia serrata*. Biomed Pharmacother. 2021;144:112-302.
21. Oliveira PL, Genta FA. Blood digestion in triatomine insects. Triatominae the Biology of Chagas Disease Vectors: Springer; 2021; 265-284.
22. Olivia NU, Goodness UC, Obinna OM. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Future J Pharm Sci. 2021;7(1):1-5.
23. Vargas S, Ndjoko Ioset K, Hay AE, Ioset JR, Wittlin S, Hostettmann K. Screening medicinal plants for the detection of novel antimalarial products applying the inhibition of *β*-hematin formation. J Pharm Biomed Anal. 2011;56(5):880-886.
24. Oluyemi WM, Samuel BB, Kaehlig H, Zehl M, Parapini S, D'Alessandro S, et al. Antiplasmodial activity of triterpenes isolated from the methanolic leaf extract of *Combretum racemosum* P. Beauv. J Ethnopharmacol. 2020;247:112203.
25. Shakya M, Shrestha PK, Briski KP. Hindbrain 5'-Adenosine Monophosphate-activated Protein Kinase Mediates Short-term Food Deprivation Inhibition of the Gonadotropin releasing Hormone Luteinizing Hormone Axis: Role of Nitric Oxide. Neuroscience. 2018;383:46-59.
26. Mamede L, Ledoux A, Jansen O, Frédérich M. Natural phenolic compounds and derivatives as potential antimalarial agents. Planta Med. 2020;86(09):585-618.
27. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. Int J Mol Sci. 2018;19(6):1578.
28. Maude RJ, Mercado CEG, Rowley J, Ekapirat N, Dondorp A. Estimating malaria disease burden in the Asia-Pacific. Wellcome Open Res. 2019;4(59):59.
29. Sathish V, Prakash Y. Sex differences in pulmonary anatomy and physiology: implications for health and disease. Sex differences in physiology: Elsevier; 2016;89-103.
30. Dai J, Mumper RJ. Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-7352.
31. Oghenejoboh UM, Nkop JN. Chemical composition, cytotoxicity and antioxidant activities of essential oils of *Parquetina nigrescens* (Afz.) bullock from ibadan, Nigeria. The Pharm Chem J. 2018;5(5):99-104.
32. Bhardwaj M, Sali VK, Mani S, Vasanthi HR. Neophytadiene from Turbinaria ornata Suppresses LPS-Induced Inflammatory Response in RAW 264.7 Macrophages and Sprague Dawley Rats. Inflamm Res. 2020;43(3):937-950.
33. Prabakaran R, Joseph B, Pradeep PN. Phyto medicinal compounds from *Urginea indica* Kunth: A synthetic drugs potential alternative. Br J Pharm Res. 2016;11(5):1-9.
34. Endo EH, Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP. Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*. Microbiol Res. 2010;161(7):534-540.
35. Christen V, Faltermann S, Brun NR, Kunz PY, Fent K. Cytotoxicity and molecular effects of biocidal disinfectants: quaternary ammonia, glutaraldehyde, poly hexamethylene biguanide hydrochloride PHMB and their mixtures *in vitro* and in zebrafish *Eleuthero embryos*. Sci Total Environ. 2017; 586:1204-1218.
36. El-Dean AMK, Abd-Ella AA, Hassanien R, El-Sayed MEA, Abdel-Raheem SA. Design, Synthesis, Characterization, and

**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

Insecticidal Bioefficacy Screening of Some New Pyridine Derivatives. ACS omega. 2019;4(5):8406-8412.
37. Faria JV, Vegi PF, Miguita AGC, Dos Santos MS, Boechat N, Bernardino AMR. Recently reported biological activities of pyrazole compounds. Bioorg Med Chem. 2017;25(21):5891-903.
38. Martelli G, Giacomini D. Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. Eur J Med Chem. 2018;158:91-105.