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PHYTOCHEMICAL PROFILING, ANTIOXIDANT, ANTITYROSINASE AND ANTIPLASMODIAL ACTIVITIES OF CHLOROFORM AND N-BUTANOL STEM FRACTIONS OF *PARQUETINA NIGRESCENS* (AFZEL.) BULLOCK: A COMPARATIVE STUDY

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ABSTRACT

The therapeutic potential of the solvent partitioned fractions of *Parquetina nigrescens* (Afzel.) Bullock was evaluated using an integrated approach that included phytochemical screening, GC-MS analysis, antioxidant, antityrosinase, antimalarial, and cytotoxicity assays. The methanolic extract of the stem was subjected to successive partitioning using chloroform and n-butanol solvents. The phytochemical screening of the chloroform and n-butanol fractions showed the presence of alkaloids, flavonoids, phenolic, and terpenoid compounds, whereas the n-butanol fraction was predominantly rich in tannins and saponins. The GC-MS analysis identified 33 and 8 compounds from the chloroform and n-butanol fractions, respectively, which included fatty acid methyl esters, diterpenoids, and hydrocarbons. The fractions demonstrated moderate DPPH radical scavenging activity (IC_{50}: 71.25 ± 1.05 µg/mL for chloroform fractions; 66.59 ± 1.40 µg/mL for n-butanol fractions) and nitric oxide scavenging activity (IC_{50}: 71.45 ± 0.86 µg/mL for chloroform fractions; 73.17 ± 1.42 µg/mL for n-butanol fractions). The n-butanol fraction exhibited strong tyrosinase inhibition (IC_{50}: 54.39 ± 1.30 µg/mL), surpassing kojic acid. Both fractions showed significant activity against *Plasmodium falciparum* (IC_{50} range: 3.224–3.611 µg/mL) with cytotoxicity IC_{50} values above 30 µg/mL on HeLa cells, yielding selectivity indices greater than 10. The pharmacological relevance of the fractions and the bioassay results validate the utility of the solvent partition method for the fractionation of *P. nigrescens*. The results also show the potential of the chloroform and n-butanol fractions as lead compounds for the development of antimalarial and tyrosinase inhibitors.

Keywords: *Parquetina Nigrescens*, GC–MS, Antioxidant Activity, Tyrosinase Inhibition, Antiplasmodial Activity, Selectivity Index

INTRODUCTION

Oxidative stress plays an important role in malaria, melanogenesis disorders, and other chronic inflammatory diseases (Adase et al., 2022; Kayoda et al., 2022; Pawlowska et al., 2023; Adekilekun et al., 2025).

Malaria is an inflammatory disease that is also quite oxidative, with reactive oxygen species playing an important role both in host defense and disease pathogenesis. During malaria, immune cells such as neutrophils and macrophages produce ROS, which is part of the host's defense mechanism to fight the malaria parasite (Vasquez et al., 2021; Gomes et al., 2022; Ofori et al., 2023). Along with this, other enzymes such as xanthine oxidase are upregulated, which increases the production of ROS even further (Vasquez et al., 2021; Ty et al., 2019). The malaria parasite also produces oxidative stress by breaking down hemoglobin from infected red blood cells, releasing free heme and iron that produce free radicals. Although free radicals produced during malaria help kill the parasite, an increase in free radicals also produces tissue damage, which may lead to severe complications during malaria (Vasquez et al., 2021; Becker et al., 2004). While these free radicals are essential for controlling the infection, excessive ROS can lead to oxidative stress, causing damage to host cells and tissues and contributing to severe malaria complications (Vasquez et al., 2021; Gomes et al., 2022; Kotepui et al., 2024; Kumar et al., 2025).

Skin cancer is the most common malignancy in humans and represents a heterogeneous group of neoplasms with increasing global incidence and mortality (Apalla et al., 2017; Garbe et al., 2024). It is broadly categorized into melanoma skin cancer (MSC) and non-melanoma skin cancers (NMSCs) (Jin et al., 2024). While NMSCs are often associated with hypopigmentation disorders such as albinism, hyperpigmentation has been linked to melanoma development (Manap et al., 2021). Melanogenesis is the physiological process responsible for melanin synthesis, which protects the skin against ultraviolet radiation (UVR)-induced damage. However, excessive melanin production results in hyperpigmentation disorders such as freckles, solar lentigines, melasma, and melanoma (Manap et al., 2021; Qu et al., 2020). UVR remains the principal environmental trigger of hyperpigmentation, primarily through upregulation of tyrosinase activity. Increased tyrosinase activity leads to enhanced melanin synthesis and accumulation (Burzyńska, 2023). Other contributing factors include hormonal imbalance (pregnancy, menopause), medications, chemicals, systemic diseases (e.g., Addison's disease), genetic predisposition, skin type, and aging (Jin et al., 2024; Thawabteh et al., 2023). In plants, tyrosinase catalyzes the oxidation of tyrosine to o-quinones, which polymerize to form melanin, leading to enzymatic browning and reduced nutritional and commercial value of fruits (Moon et al., 2020; Sui et al., 2023). Reactive oxygen and nitrogen species (ROS/RNS) generated during UV exposure are normally scavenged by melanin. Under hyperpigmented conditions, excessive ROS/RNS accumulation may induce DNA damage in melanocytes and melanoma cells, thereby contributing to carcinogenesis (Cabaço et al., 2022). Dysregulated tyrosinase activity and oxidative stress are therefore central to both hyperpigmentation and melanoma progression (Hassan et al., 2023).

Tyrosinase (EC 1.14.18.1) is a copper-containing oxidase catalyzing the hydroxylation of L-tyrosine to L-DOPA and further oxidation of L-DOPA to dopaquinone, a key

intermediate in melanin biosynthesis. Over-activation of this enzyme leads to excessive melanin accumulation and oxidative browning in biological tissues. Thus, tyrosinase inhibitors are in great demand for dermatological, cosmeceutical, and food-preservation uses (Wang et al., 2025). Although effective, their synthetic inhibitors include kojic acid, which raises concerns related to instability and possible cytotoxicity prompt the continuous search for plant-derived alternatives that could be safer.

Natural products remain fundamental to drug discovery due to their structural diversity and biological specificity (Li & Lou 2018; Lahlou 2013). Medicinal plants from the Apocynaceae family are known to contain alkaloids, terpenoids, sterols and phenolic compounds with diverse pharmacological properties (Patil 2023; Yadav & Joshi 2024). *P. nigrescens* (Afzel.) Bullock is widely used in West African traditional medicine for managing anemia, infections, inflammatory conditions and metabolic disorders (Adase et al., 2022).

Therefore, natural antioxidants and enzyme inhibitors are important therapeutic candidates. Tyrosinase inhibitors are clinically relevant in dermatology and cosmetic science, while the global burden of malaria necessitates continued discovery of novel antiplasmodial agents due to emerging drug resistance.

Although crude extracts of *P. nigrescens* have been reported to exhibit biological activity, comprehensive studies correlating solvent-partitioned fractions, GC – MS metabolite distribution, antioxidant capacity, enzyme inhibition, and parasite selectivity are limited. This study evaluates chloroform and n-butanol stem fractions to determine their phytochemical composition and pharmacological potential.

MATERIALS AND METHODS

Plant Collection and Authentication

The stem of *P. nigrescens* were collected in Osara, Kogi State, GPR: Latitude N $7^0$40’ 34.386” and E $6^0$24’ 44.892” in the month of 2023. The plants were authenticated and identified by Mr Namadi Sanusi, a botanist in the Department of Biological Science, Ahmadu Bello University ABU Zaria Nigeria., and voucher specimens (number) VN: ABU09881 were prepared and deposited in the university herbarium.

Extraction and Fractionation

800 g of powdered stem material was extracted with methanol by maceration. The concentrated methanol extract was suspended in distilled water and successively partitioned with chloroform and n-butanol. Each fraction was concentrated under reduced pressure and stored at 4° C.

Phytochemical Screening

Standard qualitative procedures were used to detect alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides and carbohydrates (Shaikh and Patil, 2020; Yinusa 2020; Shehu et al., 2023).

Gas Chromatograph-Mass Spectrometry (GC-MS)

GC-MS analysis was performed to identify the bioactive components of the chloroform and butanol, stem extracts. The system used was an Agilent 7890A GC system hyphenated to a 5975C Inert MSD with a triple-axis detector. Helium was used as the carrier gas with a flow rate of 1.8 mL/min. The separation was carried out on a capillary column (Agilent 19091-433HP-5Ms) with a length of 30 m, an internal diameter of 0.25 µm, and a film thickness of 0.25 µm. The temperature program was initiated at 50 °C for 2 minutes, ramped to 100 °C at a rate of 20 °C/min, and then increased to 250 °C at a rate of 20 °C/min, holding for 5 minutes. The total run time was 19 minutes. Compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NISTII) library.

Antiradical Activities

DPPH Antioxidant Assay

The antioxidant activity of the extracts was determined using the DPPH radical scavenging assay. 0.1 mM working solution of DPPH in Methanol was prepared. 1mg/ml of the sample was prepared in appropriate solvent. The concentration of the samples was varied to 100-500 µg/mL by serial dilution. The reaction mixture contained 1000 µL of the sample and 500 µL of DPPH reagent. The mixture was allowed to incubate at room temperature for 30 min in dark. The absorbance of the reaction mixture was taken at 518 nm against the reagent blank, methanol. The control involved methanol and DPPH reagent. Ascorbic acid was used as standard to compare the % inhibition. Calculation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance Ctrl} - \text{Absorbance Sample}}{\text{Absorbance Ctrl}} x\ 100 \quad (1)$$

Nitric Oxide Antioxidant Assay

The nitric oxide (NO) scavenging activity was determined by measuring the reduction of nitrite ions produced from the spontaneous generation of NO from sodium nitroprusside (SNP). This assay is based on the principle that NO, generated from an aqueous solution of SNP at physiological pH, interacts with oxygen to produce nitrite ions

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which can be measured by Griess reaction. Briefly, the reaction mixture contained 2 mL of 10mM sodium nitroprusside prepared in 10 mM phosphate buffered saline (pH 7.4) and 0.5 mL of different concentrations of samples (100-500 µg/mL). The mixture was incubated at 25°C for 1hr. 0.5 mL of the incubated solution was withdrawn and mixed with 0.5 mL of Griess reagent. The mixture was incubated again at room temperature for 30 minutes in the dark and its absorbance measured at 546 nm against blank. The blank contained phosphate buffered saline only while the control was all reagent without the extracts. Ascorbic acid or rutin was used as standard. The concentration of nitrite formed was derived from a regression analysis using serial dilutions of sodium nitrite as a standard. The percentage inhibition of NO radical was calculated as follow:

$$\% \text{ inhibition of NO radical } = \frac{\text{Ao} - \text{A1}}{\text{Ao}} x\ 100 \quad (2)$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the extract or standard.

Antityrosinase Assay

The anti-tyrosinase activity of the extracts was determined using L-dopa as a substrate and kojic acid as a standard according to the modified method of Wuttisin et al. certainly. (2017). 0.3 mL of tyrosinase enzyme (50 mg/mL) was preincubated with various concentrations of the standard inhibitor kojic acid and the extracts (0.5 mL) in 50 mM phosphate buffer, pH 6.8, for 5 minutes at 37 °C. L-opa (25 mM, 0.30 mL) was added to the reaction mixture and incubated at 37 °C for 10 min. The enzyme reaction was measured for the formation of DOPA-chromium by measuring the absorbance at 475 nm. The percent inhibition of tyrosinase activity was calculated using the percent inhibition equation.

$$\% \text{ inhibition} = \frac{\text{A475 nm control} - \text{A475 nm sample}}{\text{A475 nm control}} x\ 100 \quad (3)$$

The A475 nm control is the absorbance of the control solution without kojic acid (or extracts) and a 475 nm sample is the absorbance of the solution with kojic acid (or extract). The degree of inhibition was expressed as a percentage of the concentration required to achieve $_{50}$% inhibition (IC_{50}).

Antimalarial and Cytotoxicity Assays

Parasite viability was measured using parasite lactate dehydrogenase (*pLDH*) activity according to the described method (Makler *et al*., 1993). Chloroquine (Sigma Aldrich) was used as positive control. Screening of the samples against malaria parasites, which were added to the parasite cultures in 96-well plate and incubated for 48 h in a 37ºC CO_2 incubator, was carried out at a concentration of $_{50}$ µg/mL. After the expiration of 48 h, the plate was removed from the incubator. 20 µL of the culture was removed from each well and added to 125 µL of a mixture of Malstat and NBT/PES solutions in a fresh 96-well plate. These solutions were used to determine the activity of the parasite lactate dehydrogenase (*pLDH*) enzyme in the cultures. A purple product was formed when pLDH was present, and this product could be quantified in a 96-well plate reader at an absorbance of 620 nm (Abs620). The Abs 620 reading in each well was thus a sign of the pLDH activity and number of parasites in that well. The cytotoxicity were estimated against HeLa (human cervix adenocarcinoma) (Hongbao, 2017). Stock solutions extracts (20 mg/mL) were prepared in DMSO and later diluted with culture medium to 50 µg/mL and were incubated in 96- well plate containing HeLa (human cervix adenocarcinoma) cells for about 48 h. The amounts of cells that were able to outlive exposure to the drugs were also established via resazurin-based reagent and reading resorufin fluorescence in a multi-well plate reader. The obtained results were articulated as % viability (the resorufin fluorescence in compound-treated wells compared to untreated controls). This test was carried out in duplicate wells and standard deviation was generated for the targeted compounds. The results for the cytotoxicity assay were also expressed as % cell viability (obtained from fluorescence reading in treated wells versus untreated control wells). Emetine (which induces cell apoptosis) was employed as a positive standard drug.

Selectivity Index

Selectivity Index (SI) was calculated as:

Selectivity Index (S.I.) = IC_{50} (HeLa cells) / IC_{50} (Plasmodium)

Statistical Analysis

All experimental data were analyzed using GraphPad Prism Version 10.2.0 (392). All experiments were performed in triplicate, and the results were presented as the mean ± standard error of the mean (SEM). The IC_{50} values were determined by performing a nonlinear regression of the percentage inhibition against the extract concentration. A one-way analysis of variance (ANOVA) was employed for multiple comparisons of the IC_{50} values, with all analyses performed at a 95% confidence level.

RESULTS AND DISCUSSION

Percentage Yield and Qualitative Phytochemical Analysis of the Fractions

The percentage yield for chloroform, and n-butanol fractions is 0.5%, and 0.4 % for respectively. The result of the qualitative phytochemical analysis of the fractions *P. nigcresens* stem is shown in Table 1.

Table 1: Qualitative Phytochemical Composition of *P. nigcresens* Stem

Phytochemicals	Chloroform Fraction	n- Butanol fraction
Alkaloids	+	+
Flavonoids	+	+
Phenolic	+	+
Tannins	-	+
Saponin	-	+
Steroid	+	-
Cardiac glycoside	-	-
Glycosides	-	-
Terpenoids	+	+
Carbohydrates	+	+
Resin	-	-
Phlobatannis	-	-
Polysterols	-	-

Key: + (absent), - (absent)

The phytochemical detected is shown in Table 1. The chloroform fraction showed a slightly wider profile, with alkaloids, carbohydrates, flavonoids, phenolics, steroids, and terpenoids, while the remaining substances such as tannins, saponin, cardiac glycosides, general glycosides, resins, phlobatannins, and polysterols were all negative. However, the presence of alkaloids is significant, since such compounds were previously identified with the analgesic and anti-inflammatory properties reported to exist within *P. nigrescens*. Alkaloids are commonly known to be present within the species, offering healing, antidiabetic, and other pharmacological properties to the plant (Ogbole & Ajaiyeoba, 2010). This suggests that the presence of alkaloids is expected to be observed in the organic fraction, since it is likely that the alkaloids exist in the free-base form, thus increasing the solubility within organic solvents. Oyebamiji et al. (2025) identified the presence of tannins, saponin, flavonoids, alkaloids, and anthraquinones in the plant leave extract. The phytochemical profile of the n-butanol fraction yielded positive results for the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and steroids, but glycosides, steroid, resin, phlobatannis and polysterols were not present. The result suggests that the stems of *P. nigrescens* have high phenolic content. Literature on specific n-butanol fractions of *P. nigrescens* is limited, but its high polarity makes it suitable for extracting glycosides.

GC-MS Analysis of the Fractions

The GC-MS analysis chloroform and n-butanol extracts of *P. nigcresens* stem revealed the presence 33, and 8, compounds respectively. Their retention times (RT), identified compounds, names and structures, molecular weights, molecular formulas, and percentage peak areas are tabulated in Tables 2, and 3 respectively.

Chloroform Fraction

The chloroform faction revealed about thirty-three bioactive components; the total percentage composition was 99.71%. This medium non polar fraction shows a mixture of long chain alkanes/aldehydes, fatty acids/esters, isoprenoids (phytol, neophytadiene), and phthalates. The Key constituents of this extract include; Pentadecanoic acid, 14 methyl, methyl ester – 18.87%.; hexadecanal – 8.38%; pentadecanal – 3.03%; tetradecanal – 3.79%; 9-Octadecenoic acid, methyl ester – 7.66%; methyl stearate – 7.47%; Methyl-10 trans,12-cis octadecadienoate & 9,12-octadecadienoic acid – ~10.4% combined; phytol – 4.80%; neophytadiene – 1.58%; n-hexadecanoic acid – 1.76%; bis (2 ethylhexyl)phthalate – 2.14% (Table 2). The pattern of C14–C18 fatty acids/esters again supports antioxidant, anti-inflammatory and antimicrobial contributions, matching evidence that hexadecanoic and octadecadienoic acids are antioxidant, antibacterial and antifungal and may protect against oxidative and metabolic disorders. The presence of both free n-hexadecanoic acid and its esters is important because palmitic acid has been repeatedly noted in plant fractions with significant enzyme inhibition (α amylase/α glucosidase) and antimicrobial activity (Babatunde & Olusola, 2024; Hassan et al., 2022). Phytol derived diterpene with documented antioxidant, anti-inflammatory, hypolipidemic and nephroprotective actions; in *P. nigrescens* it has been specifically highlighted and studied for protection against renal damage in diabetic models (Adase et al., 2022). Neophytadiene is commonly associated with anti-inflammatory and antimicrobial potentials in GC–MS characterized plant extracts (Babatunde & Olusola, 2024; Adoga et al., 2019). Long chain hydrocarbons and aldehydes often act as inert matrix or fragrance like components, although some have mild antimicrobial or antioxidant actions (Sharmin et al., 2025; Hassan et al., 2022). Bis-(2 ethylhexyl) phthalate are frequently detected as a ubiquitous plasticizer contaminant in GC–MS runs (Sharmin et al., 2025; Hassan et al., 2022). Although some reports ascribe antimicrobial or antioxidant actions to phthalates, they are better interpreted here as processing contaminants rather than genuine plant metabolites. Phytol/neophytadiene content aligning with antioxidant, anti-inflammatory, antimicrobial and hypolipidemic/antidiabetic activities, and is consistent with chloroform extracts of *P. nigrescens* and other plants showing antimicrobial and cytotoxic potency (Babatunde & Olusola, 2024; Sharmin et al., 2025; Shettar & Hiremath, 2024; Olaleye et al., 2021).

n-Butanol Fraction

The n-butanol fraction contained a total of eight bioactive components making up 99.9% of the total fraction. Despite the more polar solvent, is dominated by fatty acid methyl esters which include; Hexadecanoic acid, methyl ester – 42.14%; Methyl stearate – 29.44%; 9-Octadecenoic acid methyl ester – 17.82%; Oleic acid – 1.58% together, these four account for >90% of the fraction (Table 3). This fraction essentially concentrates palmitate, stearate and oleate methyl esters, which are repeatedly associated with antioxidant, anti-inflammatory, antibacterial, antifungal and anticholesterolemic effects (Babatunde & Olusola, 2024; Tran et al., 2023; Hassan et al., 2022). Cis vaccenic and related C18 unsaturated fatty acids are known to improve insulin sensitivity and lower LDL cholesterol (Hassan et al., 2022), consistent with the antidiabetic and antilipidemic activities attributed to *P. nigrescens* (Adase et al., 2022; Ayoola et al., 2024; Bamisaye et al., 2023). n-butanol fraction is a dense source of bioactive fatty acid esters, and can be interpreted as a major contributor to antioxidant, anti-inflammatory, hypolipidemic and antidiabetic actions of the methanol extract, especially via modulation of lipid metabolism and oxidative stress (Huang et al., 2024; Ayoola et al., 2024; Bamisaye et al., 2023; Hassan et al., 2022).

Table 2: GC-MS Profiling of the Chloroform Fractions of the Methanol Extract of *Parquetina nigrescens*

S/N	Bioactive Compounds	Molecular formula & mass	RT (min)	% Composition
1	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$206.17	9.24	1.08
2	1-Nonadecene	$C_{19}H_{38}$266.30	9.90	1.08
3	Hexacosane	$C_{26}H_{54}$366.42	9.96	1.67
4	Heptadecanal	$C_{17}H_{34}O$254.26	11.12	0.74
5	Methyl 11-oxo-9-undecenoate	$C_{12}H_{20}O_3$212.14	11.18	0.73
6	3-Eicosene, (E)-	$C_{20}H_{40}$280.31	11.75	1.44
7	Heptadecane, 8-methyl-	$C_{18}H_{38}$254.30	11.80	1.20
8	Tetradecanal	$C_{14}H_{28}O$212.21	12.00	3.79
9	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$256.24	12.06	0.95
10	Neophytadiene	$C_{20}H_{38}$278.5	12.15	1.58
11	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$268.28	12.24	2.49
12	Cyclohexane, 1,1'-(1,4-butanediyl)bis-	$C_{16}H_{30}$222.23	12.38	0.84
13	9-Heptadecanone	$C_{17}H_{34}O$254.26	12.49	0.57
14	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$270.26	12.91	18.87
15	n-Hexadecanoic acid	$C_{16}H_{32}O_2$256.24	13.32	1.76
16	5-Eicosene, (E)-	$C_{20}H_{40}$280.31	13.42	1.35
17	Tritetracontane	$C43H_{88}$604.69	13.47	0.93
18	Hexadecanal	$C_{16}H_{32}O$240.25	13.69	8.38
19	1-Octadecene	$C_{18}H_{36}$252.28	14.18	0.74
20	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$294.26	14.26	6.86
21	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$296.27	14.30	7.66

S/N	Bioactive Compounds	Molecular formula & mass	RT (min)	% Composition
22	Phytol	$C_{20}H_{40}O$296.31	14.41	4.80
23	Methyl stearate	$C_{19}H_{38}O_2$298.29	14.49	7.47
24	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$312.30	14.92	4.77
25	Pentadecanal	$C_{15}H_{30}O$226.23	15.22	3.03
26	17-Pentatriacontene	$C_{35}H_{70}$490.55	15.54	0.94
27	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$326.32	16.00	2.03
28	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$280.24	16.24	3.59
29	Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$340.33	16.51	1.84
30	Heptadecanal	C_{17}H34O254.26	16.94	2.09
31	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$242.26	17.65	0.27
32	Methyl 20-methyl-heneicosanoate	$C_{23}H_{46}O_2$354.35	18.09	2.03
33	Bis(2-ethylhexyl)phthalate	$C_{24}H_{36}O_4$388.26	18.40	2.14
Total				99.71

Table 3: GC-MS Profiling of the n-Butanol Fractions of the Methanol Extract of *Parquetina nigrescens*

S/N	Bioactive Compounds	Molecular formula & mass	RT (Min)	% Composition
1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ 270.26	12.92	42.14
2	13-Oxabicyclo[10.1.0]tridecane	$C_{12}H_{22}O$ 182.17	13.46	3.13
3	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$ 296.27	14.32	17.82
4	Methyl stearate	$C_{19}H_{38}O_2$ 298.29	14.50	29.44
5	Oxirane, tridecyl-	$C_{15}H_{30}O$ 226.23	15.25	2.68
6	Methyl trans-9-(2-butylcyclopentyl)nonanoate	$C_{19}H_{36}O_2$ 296.27	16.02	1.33
7	1,5,9,13-Tetradecatetraene	$C_{14}H_{22}$ 190.17	18.16	1.87
8	Oleic acid	$C_{18}H_{34}O_2$ 282.5	18.43	1.58
Total				99.99

Antioxidant Analysis of the Fractions

Antioxidant activity is one of the most important biochemical properties of plant metabolites that reflects the ability of extracts to counteract oxidative stress due to reactive oxygen and nitrogen species. Oxidative imbalance underlies many pathological conditions, among which are malaria, cancer, and skin disorders.

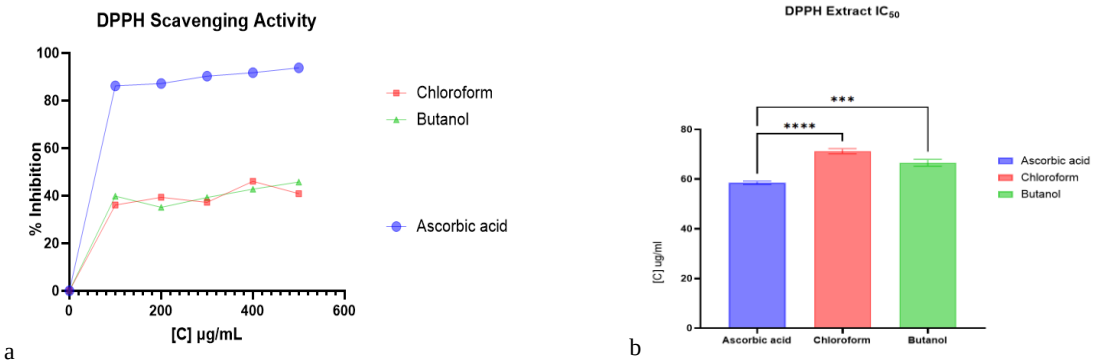
DPPH Inhibitory Activity of the Fractions

All *P. nigrescens* fractions manifested quantifiable radical-scavenging activity. The fractions show a dose dependent inhibition with Chloroform and n-butanol fractions both have significantly IC_{50} values (71.25±1.05 and 66.59±1.40 µg/mL), than ascorbic acid (58.52 µg/mL) the standard ($p < 0.0001$) , indicating moderate antioxidant activity, but weaker than the standard (Table 4, Figure 1). Phenolics and flavonoids are established radical scavengers and were detected in both fractions, supporting the DPPH activity. GC–MS analysis indicate that chloroform and n-butanol fraction is enriched fatty acids/esters (palmitic, stearic, oleic, linoleic acids), This is consistent with work on *P. nigrescens* leaves, where methanol/ethanol extracts show IC_{50} values around 59–87 µg/mL in DPPH, suggesting moderate–good radical scavenging driven by phenolics and flavonoids (Olaleye et al., 2021; Daramola et al., 2025). The presence of flavonoids, tannins and other phenolics in *P. nigrescens* is well documented and linked to its antioxidant properties in vitro and in vivo (Olaleye et al., 2021; Daramola et al., 2025; Bamisaye et al., 2023; Ajayi et al., 2021).

The DPPH test results (Table 4 & Figure 1), shows the percentage of inhibition (%) of the *P. nigrescens* stem fractions and IC_{50}. The active controls ascorbic acid was used.

Table 4: DPPH Radical-scavenging Activity and IC_{50} of the Fractions

Conc. (µg/mL)	Chloroform	n-Butanol	Ascorbic Acid
100	36.13 ± 0.36	39.84 ± 39.84	86.24 ± 0.57
200	39.32 ± 0.28	35.09 ± 35.09	87.18 ± 0.82
300	37.24 ± 0.18	39.24 ± 39.24	90.30 ± 0.70
400	46.07 ± 0.28	42.80 ± 42.80	91.72 ± 0.38
500	40.88 ± 0.38	45.77 ± 45.77	93.78 ± 0.38
IC_{50}	71.25±1.05	66.59±1.40	58.52±0.68



a b

Figure 1: (a) Percentage Inhibition and (b) IC_{50} Value of the DPPH Activity of the Fractions

Nitric Oxide Inhibitory Activity of the Fractions

Both fractions of *P. nigrescens* exhibited measurable inhibition of nitric-oxide radicals. As concentration of of chloroform and n-butanol increases so inhibition increases with IC_{50} values 71.45±0.86 and 73.17±1.42 µg mL^{-1} for chloroform and n-butanol fractions. For nitric oxide scavenging, rutin was the standard (IC_{50} = 73.19±0.85 µg/mL). The activity of chloroform and n-butanol were similar or comparable to rutin with no significant difference (Table 5 & Figure 2).

The Nitric oxide test results (Table 5 & Figure 2), shows the percentage of inhibition (%) of the *P. nigrescens* stem fractions and IC_{50}. The active controls rutin was used.

Table 5: Nitric Oxide Scavenging Properties and IC_{50} of the Fractions

Conc. (µg/mL)	Chloroform	n-Butanol	Rutin
100	32.80 ± 0.60	25.98 ± 1.14	55.53 ± 0.42
200	34.71 ± 0.19	26.90 ± 0.82	59.45 ± 0.84
300	37.85 ± 0.60	31.65 ± 0.93	64.73 ± 0.90
400	38.62 ± 0.33	31.88 ± 0.78	68.39 ± 0.92
500	42.61 ± 0.29	33.56 ± 0.98	73.29 ± 0.78
IC_{50}	71.45±0.86	73.17±1.42	73.19±0.85

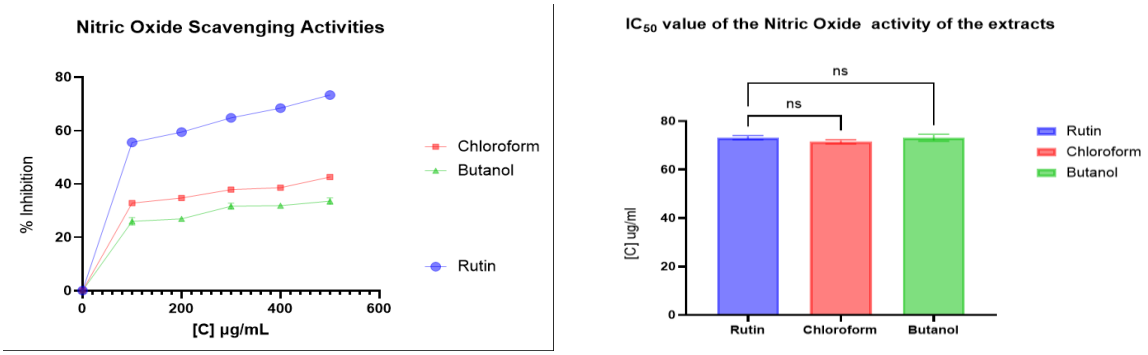


Figure 2: (a) Percentage Inhibition and (b) IC_{50} Value of the Nitric Oxide Activity of the Fractions

Anti-tyrosinase Inhibitory Activity of the Fractions

In the present study, L-DOPA as substrate was used spectrophotometrically to evaluate the inhibitory effects of five solvent fractions of *P. nigrescens* stem extract. The obtained results of % inhibition and IC_{50} (µg mL^{-1}) are given in Table 6, Figure 3. Both fractions were dose-dependent inhibitors of tyrosinase, but they exhibited various potencies. Kojic acid (IC_{50} = 78.10 µg/mL) served as the standard. n-butanol showed better or comparable antityrosinase activity. n-butanol (IC_{50} = 54.39 µg/mL) is significantly potent than kojic acid while chloroform 73.83 µg/mL (p = 0.0143, slightly better than kojic). This indicates very strong tyrosinase inhibitory activity, which may be due to the presence of phenolics and flavonoid detected across the fraction. Plant phenolics and flavonoids are well known tyrosinase inhibitors, and other species rich in such compounds (e.g., Morina persica, various phenolic rich extracts) show similar potent antityrosinase effects (Zeitoun et al., 2020; Özgen et al., 2024; Tilkat et al., 2021). *P. nigrescens* leaves contain flavonoids, tannins, and other phenolics (Olaleye et al., 2021; Daramola et al., 2025; Bamisaye et al., 2023).

Extract containing flavonoids and phenolics, are widely documented as competitive or mixed inhibitors of tyrosinase due to their ability to chelate the active site copper and mimic phenolic substrates (Yadav & Pandey 2025; Ebrahimi et al., 2025; Panzella & Napolitano 2019)

n-butanol is dominated by hexadecanoic acid methyl ester, 9 octadecenoic acid methyl ester, methyl stearate, with some oleic acid. Fatty acid rich fractions alone are rarely as potent as kojic acid, so the strong activity seen for n-butanol suggests additional polar phenolics or triterpenoids not fully visible in GC–MS, consistent with many phenolic rich butanol fractions that show good tyrosinase inhibition (Zeitoun et al., 2020; Hsu et al., 2022). while chloroform contain phytol and diterpenoids. Phytol and similar diterpenoids are repeatedly found in plant extracts with anti-tyrosinase activity and can contribute to general enzyme inhibition and antioxidant effects (Rosa et al., 2024; Ko & Cho 2018). Also, Terpenoids

have antioxidant and anti-inflammatory properties that may contribute to tyrosinase inhibition by reducing NO and ROS mediated up regulation of melanogenesis (Prakash 2017; Corradi et al., 2018)

Biologically, such potent antityrosinase activity suggests potential for skin lightening, anti-browning, or anti-melanogenesis applications, although in vivo safety and efficacy would still need to be established (Zeitoun et al., 2020; Özgen et al., 2024; Tilkat et al., 2021). The chemical benchmark, kojic acid, inhibits tyrosinase by forming a stable chelate with the active-site copper ions through its 5-hydroxypyran-4-one structure. It is remarkable that all fractions matched or even outperformed such potency of kojic acid, demonstrating that *P. nigrescens* fractions contain components with similar efficiency regarding metal binding. In contrast with kojic acid, which can suffer photodegradation and thus favors irritation, terpenoids and sterols have excellent photostability and are less cytotoxic (Lajis et al., 2012). Thus, these natural inhibitors are safer alternatives for cosmetic and pharmaceutical formulations.

The antityrosinase test results (Table 6 & figure 3), shows the percentage of inhibition (%) of the *P. nigrescens stem* fractions and IC_{50}. The active controls kojic acid was used

Table 6: Anti-tyrosinase Scavenging Properties and IC_{50} of the Fractions

Conc. (µg/mL)	Chloroform	n-Butanol	Kojic Acid
100	20.45 ± 0.27	60.50 ± 0.55	53.14 ± 1.80
200	21.43 ± 0.27	61.26 ± 0.40	59.20 ± 0.40
300	23.81 ± 0.61	60.71 ± 0.27	62.55 ± 0.55
400	25.32 ± 0.27	61.47 ± 0.40	66.23 ± 0.27
500	26.84 ± 0.15	52.49 ± 12.09	75.97 ± 1.59
IC_{50}	73.83±1.84	54.39±1.30	78.10±2.52

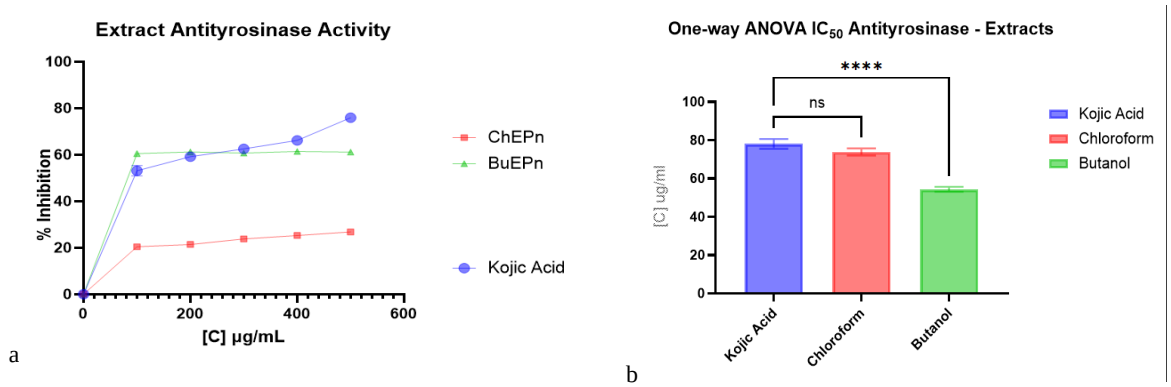


Figure 3: (a) Percentage Antityrosinase Inhibition and (b) IC_{50} Value of the Anti-tyrosinase Activity of the Fractions

Antimalarial and Cytotoxicity Result

The two solvent fractions of *P. nigrescens* had detectable inhibition against *Plasmodium falciparum* as in Table 7.. Such values are within an activity range considered significant for extracts of plants (IC_{50} < 5 µg mL^{-1}) (Larayetan et al., 2019). As shown in Figure 4, both fractions exerted a concentration-dependent decrease in *P. falciparum* viability comparable to the reference drug, chloroquine. The reference drug, chloroquine, gave an IC_{50} of 0.0118 µM, representing the classical nano-molar potency associated with its specific inhibition of heme polymerization. The chloroform exhibited the least activities with IC_{50} value of 3.611 µg mL^{-1} while the n-butanol fraction was the most potent, with an IC_{50} value of 3.224 µg mL^{-1} (Figure 7). Both Fraction, which are rich in fatty acid ester and aldehyde, exhibited fair activity.

Table 7: *pLDH* (Malaria) assay – %Viability and IC_{50} of the Fractions

log (ug/ml)	Chloroform	Butanol	Log	Chloro-quine
2.40	-0.76 ±4.72	11.92 ±1.56	0.00	-3.55±6.36
1.92	-20.75 ±0.56	-16.23 ±7.95	-0.48	5.65 ±10.30
1.44	-2.03 ±1.05	-7.94 ±5.31	-0.95	-2.80 ±5.21
0.97	15.23 ±0.26	12.39 ±3.10	-1.43	3.17 ±15.41
0.49	55.17 ±6.41	59.04 ±27.97	-1.91	50.73 ±15.88
0.01	116.71 ±1.34	107.72 ±12.39	-2.39	105.32±3.26
-0.46	110.97 ±9.43	122.08 ±11.05	-2.86	111.36 ±8.39
-0.94	103.19 ±5.56	114.16 ±3.67	-3.34	106.77 ±6.29
IC_{50}	3.611	3.224		0.01182

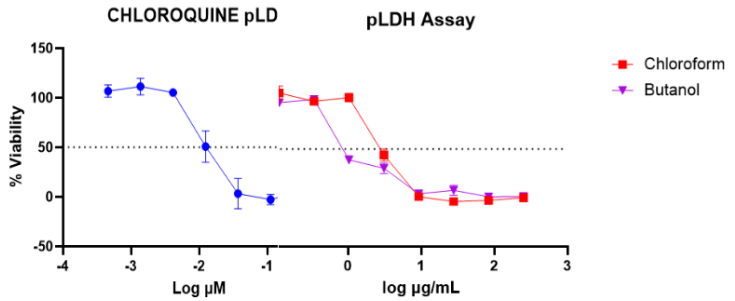


Figure 4: Dose Response Curve for *pLDH* (Malaria) Assay – IC_{50} of Fractions

HeLa-cell cytotoxicity tests yielded IC_{50} values of: SO_2 = 57.40, and SO_4 = 41.07 µg mL^{-1} (Table 8). All exceeded the National Cancer Institute's non-toxicity threshold (> 30 µg mL^{-1}). Thus, all fractions are considered non-toxic or weakly cytotoxic. The order of toxicity suggests that polar extracts contain more reactive molecules responsible for mild cellular stress.

Table 8: Cytotoxicity Assay – IC_{50}

Compound (uM for Emetine, ug/ml for Samples)	IC_{50}
Emetine	0.01864
Chloroform- SO_2	57.4
n-Butanol - SO_4	41.07

Chloroform and n-butanol fractions all recorded Selectivity Index (S.I.) > 10 (Table 9), implying high parasite specificity and acceptable safety for human cells. The values confirm that triterpenoid and sterol-rich fractions act on parasite-specific targets rather than general mammalian membranes.

Table 9: Comparison of Antiplasmodial Activity, Cytotoxity and Selective Index of *Parquentina nigrescens* Fractions (SO2 & SO4)

Fraction	IC_{50} (antiplas.)	IC_{50} (HeLa)	S.I.	Interpretation
Chloroform	3.611	57.40	≈ 15.9	Selective
n-Butanol	3.224	41.07	≈ 12.7	Selective

CONCLUSION

The findings demonstrate that stem fractions of P. nigrescens as a potent antityrosinase agent, with all fractions matching or surpassing the reference inhibitor kojic acid, thereby validating the ethnomedicinal use of this species for skin-related disorders. The presence of phenolics and flavonoids across all fractions, together with terpenoids, and sterols, suggests that multiple chemical classes act cooperatively to modulate melanogenesis through copper chelation, substrate mimicry and indirect antioxidant/anti-inflammatory mechanisms. GC-MS profiling linked the potent activity of non-polar and polar fractions to triterpenoid and sterol scaffolds, which are characterised in the literature by good skin permeability and low cytotoxicity, supporting their suitability as safer alternatives to kojic acid whose clinical use is limited by photodegradation and irritation. Overall, the study positions P. nigrescens stem as a valuable reservoir of multi-target tyrosinase inhibitors with potential applications in skin-lightening, anti-browning and anti-melanogenesis formulations. In the antiplasmodial assay, both fractions displayed significant activity against *Plasmodium falciparum* falling within the range considered active for plant extracts. Cytotoxicity evaluation against HeLa cells revealed IC_{50} values above 30 µg/mL, and selectivity indices greater than 10 confirmed parasite-specific action with acceptable safety. Overall, the findings establish that stem fractions of *P. nigrescens* possess antioxidant, antityrosinase and antiplasmodial activities. The comparative data demonstrate that metabolite distribution influenced by solvent polarity plays a critical role in determining biological potency, thereby supporting the pharmacological relevance of this traditionally used medicinal plant.

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