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Comparative Studies on the Bioactive Chemical Composition, Antioxidant and Antimalarial potentials of *picralima nitida* Leaf and Stem Bark Extracts

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Abstract

Picralima nitida has a wide range of application in Africa traditional medicine. This research was designed to comparatively evaluate the bioactive constituents, antioxidant, and antimalarial potentials of leaves and stem bark of Picralima nitida. The plant's leaves and stem bark were successfully extracted with methanol using cold extraction technique. The determination of the chemical constituents, antioxidant and antimalarial potentials were investigated using already established methods. The phytochemicals discovered in the leaves and stem bark include alkaloids, saponins, terpenoids, flavonoids, reducing sugar, tannins, phenolic compounds, glycosides, proteins and carbohydrates. The GC-MS analysis revealed 3-O-methyl-d-glucose (70.56%), phytol (5.67%), and hexanoic acid, 2-ethyl hexyl ester (13.93%), as the major components of the leaf extract while dodecanoic acid (18.34%), 9 octadecanoic acid (7.49%) and n-hexadecanoic acid (5.95%) were revealed as the stem bark extract's chief components. The antioxidant potential at 200, 100, 50, 20, 10, 5, 2 and 1 μg/mL gave an IC_{50} of 2.08 ± 1.28, 14.76 ± 10.64 and 12.48 ± 9.66 μg/mL for standard, leaf extract and stem bark extract respectively. In the in vivo anti-malarial activity, a maximum average parasitaemia suppression of 47.00 ± 5.57 and 53.34 ± 3.63 % was observed at 800 mg/kg, for the leaf extract and the stem bark extract correspondingly. The stem bark extract showed a higher average chemo-suppression compared to the leaves extract. By this study, the use of P. nitida in the treatment of malaria and other ailments associated with oxidative stress is validated.

Keywords: *Picralima nitida***,** *phytochemicals, antioxidant, antimalarial, GC-Ms*

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1. INTRODUCTION

Medicinal herbs can also be referred to as therapeutic or curative plants. Since prehistoric times, they have been recognized and used customarily in folk curative procedures. Plants are the source of numerous beneficial products that have been used for many years as tried-and-true remedies for a variety of human illnesses (Lichterman, 2004; Iyasele *et al*., 2022). Our ancestors were compelled to look into them for answers because they were seeking divine health, long life, and remedies for discomfort and pain. Plant extracts continue to be the most abundant source of novel medications, despite the fact that millions of chemical compounds with therapeutic potential are being produced in laboratories from time to time (Ogbeide *et al.*, 2018). Over the years, the public has long used medicinal plants as an alternative therapy to treat a variety of illnesses (Dutra, *et al.*, 2016). People from different cultures have benefited from all of the traditional knowledge about medicinal plants that have been passed down through the generations, particularly in rural areas or in isolated areas with low population without proper access to healthcare. The discovery of anti-inflammatory, antioxidant, antibacterial, anti-mutagenic, anti-carcinogenic, and anti-malarial medications have considerable credits to medicinal plants utilized in traditional medicine (Ogbeide *et al*., 2020a).

Phytochemicals are plant-produced compounds that defend plants against harm. They can be found in various sources like grains, fruits, veggies, nuts, and herbs, with over a thousand types identified. Key phytochemicals include carotenoids, polyphenols, isoprenoids, phytosterols, saponins, fibers, and polysaccharides (Shikov *et al*., 2022). After they are extracted from different sources, phytochemicals are crucial to the development of functional foods and nutraceuticals. These phytochemicals exhibit different affinities for solvents and tolerances to heat. The choice of solvent also impacts the quality of the extracted phytochemicals and its application in food and nutraceutical product development (Uadia *et al.*, 2023).

Picralima nitida belongs to the Apocynaceae family and genus Picralima. The Yoruba people in the southwest of Nigeria refer to it as *Abeere*, the Igbo call it *Ise-Igwe* and the Edo identifies it as *Osu* (Puch, 2021). It is found in tropical African nations like Gabon, Ivory Coast, Nigeria, and Uganda (Amaeze *et al*., 2018; Olajide *et al*., 2014). In conventional medicine, the herb is used to treat and manage diabetes, hepatitis, pneumonia, malaria, abscesses and other prevalent diseases (Erharuyi *et al*., 2014).

Cells of plants possess a sophisticated antioxidant system with both non-enzymatic and enzymatic constituents to preclude the oxidation reaction which can lead to varieties of disease such as cancer (Barua *et al.*, 2014). While most people believe medicinal herbs to be harmless, some of them can be relatively unsafe (Boukandou-Mounanga *et al*., 2015). Medicinal plant toxicity varies according to the chemical makeup of the plant. Therefore, despite the various benefits of medicinal plants, some may be harmful to users' health due to adverse effects that could arise from overdose (Tamokou and Kuete, 2014; Schultz *et al*., 2020).

Human liver and kidney issues have been connected to the use of traditional herbal remedies (Tamokou and Kuete, 2014). Several animal studies have also shown that certain medicinal plants are detrimental to certain organs of the body, and some contain several toxic components that can affect multiple systems, resulting to a clampdown of the Immune system, thereby making the body exposed to a number of diseases, including malaria (Uadia *et al.,* 2023; Oghogho *et al.,* 2022).

Despite significant advancements in parasitic diseases' treatment, malaria continues to pose a serious threat to the public healthiness in regions where the disease is common. These challenges are primarily caused by the parasites' widespread resistance to currently available anti-malaria agents, the mosquito vectors' resistance to presently available insecticides, the lack of advancement in developing vaccines against malaria, and the unfavourable side effects of conventional anti-malaria drug treatments (Oghogho *et al.,* 2022; Ogbeide *et al.,* 2018). There is a chance that three billion people could contract malaria due to *Plasmodium falciparum* and *Plasmodium vivax* infections, which cause over a million deaths annually. Thus, the necessity for novel antimalarials is highlighted by advancements and the complexity involved in creating effective vaccines. Traditional herbal medicine is the mainstay of accessible malaria treatments in developing nations, particularly in Africa. Indeed, many infectious diseases can be effectively treated with local plants, and many of these treatments rely on herbal remedies (Oghogho *et al.,* 2022).

Based on this, the study aims to examine the bioactive constituents, antioxidant and antimalarial potentials of extracts from *Picralima nitida* leaves and stem bark.

2. MATERIALS AND METHOD

Plant Sample Collection

Fresh leaves and stem bark of *Picralima nitida* were procured in Useh community Benin City. A plant scientist; Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, identified and authenticated the plant and a voucher number UBH-P424 was deposited.

Plant Preparation and Extraction

After being cleaned and cut into pieces, the fresh leaves and stem bark were allowed to air dry for three weeks at a room temperature of 30±0.5 °C. The samples, with a net weight of 1,100 g for the leaves and 900 g for the stem bark, were ground into a fine powder using a mechanical grinder in accordance with the Wokocha and Okereke (2005) method. The powdered leaves and stem bark were kept in a separate air-tight vessel and left for further investigation. An exhaustive cold maceration was done by pouring 500 g each of the samples into a glass vessel and separately mixed with 1 L of methanol and then left for 3 days with shaking, then filtered. Another 500 mL each of the methanol was added separately to the residues in both extractions vessels and left for another 3 days and then followed by filtration. This extraction was repeated using ethyl acetate solvent for both the leaf and stem bark. A rotary evaporator at 40°C was used to concentrate the filtrates to dryness. After the extracts' weights were determined, they were kept at 4 °C in a fridge-freezer until analysis. Equation 1 was used to calculate the percent yield.

$$\%\ \text{yield} = \frac{weight\ of\ extract}{weight\ of\ sample} \times 100 \qquad (1)$$

Preliminary Phytochemical Screening

The qualitative examination of the phytochemicals present in the leaf and stem bark of *P. nitida* was carried out according to the methods described by Pandey and Tripathi (2014), Uadia *et al.* (2023).

Determination of Antioxidant Potential

In this investigation, 0.1 mM methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was employed. 3.0 mL of the plant extract at concentrations of 5, 10, 25, 50, 100, and 200 μg/mL was combined with 1.0 mL of the DPPH solution using a micropipette, and the mixture was vigorously shaken, and the test tubes were left to stand for 30 minutes. Subsequently, the absorbance was recorded at 517 nm according to (Jain *et al.,* 2008). The equation (2) was used to calculate the DPPH radical scavenging potential.

DPPH radical scavenging potential (%)

$$= \frac{Absorbance\ of\ \ blank - Absorbance\ of\ test\ sample}{Absorbance\ of\ blank} \times 100 \qquad (2)$$

Plotting the % DPPH scavenging activity against sample concentration allowed for the determination of the plant extract concentration at which 50% inhibition (IC_{50}) was produced. The standard utilized was ascorbic acid (Jain *et al.,* 2003).

Experimental Animal Handling

Animal breeding facilities of the Department of Biochemistry at the University of Benin provided Swiss mice (18–24 g) of both sexes. The animals were kept in the University of Benin's Animal House division, which is part of the Department of Animal and Environmental Biology. The animals were housed in plastic cages with natural lighting and a 12:12 hour dark/light cycle at room temperature. In accordance with the National Institutes of Health's (NIH) Guide for the care and use of laboratory animals, they were fed with standard diet and water *ad libitum*. All procedures of the experiment were approved by the Ethical Review Board of Faculty of Life Sciences, University of Benin (Uadia *et al.,* 2023).

Antimalarial Studies

Inoculation of Parasite

Plasmodium *berghei* was gotten from the National Institute of Medical Research (NIMR), located in Lagos, Nigeria. First, the donor's blood's parasitaemia was determined. The mice were then killed by cervical dislocation, and the jugular vein was severed to collect blood in a Petri dish containing an anticoagulant (0.5% trisodium citrate). Based on the parasitemia of the donor mice and the number of red blood cells (RBC) in normal mice, the blood was then diluted with physiological saline (0.9%) so that 1 mL of blood contained $5x10^7$ P. *berghei*-parasitized erythrocytes. 0.1 mL of infected blood containing roughly 1×10^7 P. *berghei*-parasitized erythrocytes was administered intraperitoneally to each mouse used in the experiment (Misganaw *et al.,* 2019).

Giemsa solution preparation

The 3.5 g of Giemsa powder was dissolved in 250 mL of glycerol and 250 mL of methanol. The experimental procedure took place in a dimly lit room. For a week, the solution was stored in a dark cupboard after being poured into a dark reagent bottle (Ogbeide *et al.,* 2020b).

Phosphate buffer saline (pbs) Preparation

Distilled water was used to dissolve 10.9 g of sodium dihydrogen phosphate and 3.2 g of disodium hydrogen phosphate, and the solution was then made up to 1000 mL. Using a diluted NaOH solution, the pH of the mixture was brought to 7.2 (Ogbeide *et al.,* 2020b).

Suppressive activity evaluation of extract on early infection (4 days test)

The method adopted was according to (Knight and Peter, 1980), the antiplasmodial activity of chloroquine and extracts from leaves and stem bark against early *P. berghei* infection in mice was assessed. Twenty-five Swiss mice were injected (intraperitoneally) with *P. berghei*-parasitized erythrocytes on the first day (D1); these mice were then randomly assigned to five groups (Groups A, B, C, D, and E), each consisting of five animals, and these groups were treated for the next four days (D1–D4). Group D received no treatment, Group E received oral Chloroquine at a dose of 5 mg/kg per day, and Groups A, was according B, and C received daily doses of the extract (200, 400, and 800 mg/kg body weight of animal, respectively). Two hours after the parasite inoculation on Day 1, the required dose was given based on the animal's weight. Thereafter, it was given once daily for three more days (D2 – D4)

Parasitaemia Evaluation

Day five (D5) of the study involved the preparation of thick and thin films using mouse tail blood. Day five through day eight (D5 – D8) saw the thin blood films of infected and treated mice examined under a microscope to determine the level of parasitaemia. The thin film was fixed with methanol and both films were stained with giemsa. Oil immersion was used to mount the stained slides. In ten fields, red blood cells were counted, and the parasitized were also recorded. As shown in equation (3), the percentage of parasitaemia (Pp) in a group was computed.

$$\text{Pp} = \frac{number\ of\ parasitised\ red\ blood\ cells}{total\ number\ of\ red\ blood\ cells} \times 100 \qquad (3)$$

The following formula was used to determine the average percentage suppression for each extract dose relative to the control:

$$\text{Average \% suppression} = \frac{X - Y}{X} \qquad (4)$$

Where X is the average percentage parasitaemia of the negative control while, Y is the Average percentage parasitaemia of the treated groups (Ogbeide *et al*., 2020b).

Statistical Analysis

SPSS version 20.0 for Windows was used to analyze the data. The study's findings are presented as mean ± standard errors of the mean (SEM). One-way analysis of variance (ANOVA) was used to analyze them, and it was followed by the student-Newman Keuls posthoc test or the Bonferroni t-test. $P < 0.05$ was deemed significant.

3. RESULT AND DISCUSSION

Percentage Yield of Extract

The percentage yield for the leaves and stem bark extract of *Picralima nitida* obtained were 15.912% and 7.605% respectively. The leaves extract has a higher yield which agrees with Okorie *et al*. (2022).

Phytochemical Screening

Table 1: *Phytochemical Components of Picralima nitida leaf and stem bark*

Phytochemicals	Methanol		Ethyl Acetate	
	Leaf	Stem bark	Leaf	Stem-bark
Alkaloids	+	+	-	+
Saponins	+	+	1.	-
Terpenoids	+	-	-	+
Reducing Sugar	-	+	+	+
Flavonoids	+	-	-	+
Tannins	+	+	+	-
Phenolic Compounds	+	+	+	-
Protein	+	+	+	+
Glycosides	1.	-	+	+
Carbohydrates	+	+	+	+

+ = Present − = Not detected

The outcome of the preliminary qualitative phytochemical investigation of *P. nitida* leaves and stem bark indicate that some therapeutically active components are present; these include: alkaloids, phenolic compounds, flavonoids, reducing sugar, tannins, protein, glycoside and carbohydrate. This outcome agrees with the existing literature by Erharuyi *et al*. (2014). The phytochemical screening of the leaves shows that alkaloids, terpenoids, saponins, reducing sugar, phenolic compounds, tannins, glycoside, protein and carbohydrate are present while that of the stem bark shows that alkaloids, terpenoids, saponins, flavonoids, tannins, reducing sugar, glycosides, phenolic compounds, protein and carbohydrate are present, as shown in Table 1.

According to various studies, these biologically active components are known to exhibit different biological activities (Uadia *et al.*, 2023; Ogbeide *et al*., 2018). The presence of alkaloids in *P. nitida* increases the pharmacological importance. This is due to the fact that it has been proven scientifically that alkaloids possess antimalarial, analgesic, antidiabetic, and mildly stimulating properties (Ogbeide *et al*., 2022). Saponins reduce blood cholesterol levels by blocking the reabsorption of extra cholesterol into the bloodstream and by acting as a non-sugar component of the saponins as well as lowers the chances of cardiovascular illnesses (Ebe *et al*., 2019). Furthermore, studies have shown that saponins significantly reduce the risk of gastric ulcers by increasing the proportion of the stomach lining that is resistant to ethanol and stress-induced ulcers in laboratory animals (Aiwonegbe *et al.,* 2020). It is well known that terpenes possess cytotoxic properties. *P. nitida* leaves and stem bark contain terpenoids, which implies that an extract from them may be utilized to create anti-viral and anti-tumor drugs and treatments (Aiwonegbe and Iyasele, 2021). Flavonoids have anti-allergic, antibacterial, anti-thrombotic, anti-inflammatory and viral effects according to Ogbeide *et al.* (2022). Even while foods high in tannins often have minimal nutritional value, it has been suggested that drinking tannin-rich beverages, such as red wines and green teas, can help treat or even prevent a number of conditions, including heart disease (Daniyan *et al.*, 2018). Additionally, phytochemicals function as free radical scavengers, assisting in the elimination of highly charged oxygen molecules that are byproducts of oxygen metabolism and may offer a number of health advantages. Therefore, medicinal plants offer a promising avenue for developing new therapeutic agents to treat a variety of tropical illnesses, including those brought on by protozoa (Uadia *et al.,* 2023).

Gc-Ms Analysis of P. Nitida Leaves and Stem Bark Extracts

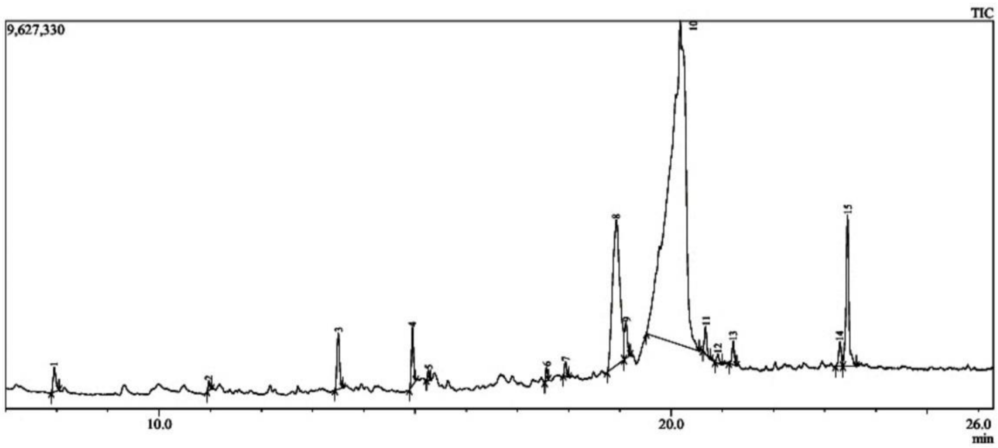

Figure 1: *GC-MS chromatogram of P. nitida leaves extract*

Table 2: *Compounds Identified in GC-MS Study of P. Nitida leaves extract*

Peak No.	Retention Time (Rt)	Name of compound	Area Percentage %	Molecular mass	Molecular formular
1	7.951	2-pentanone, 4-hydroxy-4-methyl	0.89	116	$C_6H_{12}O_2$
2	10.975	Benzyl alcohol	0.23	110	C_7H_8O
3	13.498	Benzofuran, 2, 3-dihydro	1.88	120	C_8H_8O

4	14.949	2-methoxy-4-vinylphenol	1.65	150	$C_9H_{10}O_2$
5	15.261	1-tridecene	0.22	182	$C_{13}H_{26}$
6	17.566	1-pentadecene	0.29	210	$C_{15}H_{30}$
7	17.943	3,5-Dimethoxyacetphenone	0.40	180	$C_{10}H_{12}O_3$
8	18.932	Hexanoic acid,2-ethylhexyl ester	13.93	228	$C_{14}H_{28}O_2$
9	19.124	2-butanone,4-(2,2,6-trimethylcyclohexyl)-	1.32	196	$C_{13}H_{24}O$
10	20.187	3-0-methyl-d-glucose	70.56	195	$C_7H_{14}O_6$
11	20.676	Phytol,acetate	0.82	338	$C_{22}H_{42}O$
12	20.917	3-(3,6-dimethyl-3,6-dihydro-2H-pyran-2-yl)-3	0.25	260	$C_{15}H_{16}O_4$
13	21.218	Hexadecanoic acid, methyl ester	0.66	270	$C_{17}H_{34}O_2$
14	23.304	9-octadecanoic acid, methyl ester, (E)-	0.89	296	$C_{19}H_{36}O_2$
15	23.450	Phytol	5.67	296	$C_{20}H_{40}O$
			100.00		

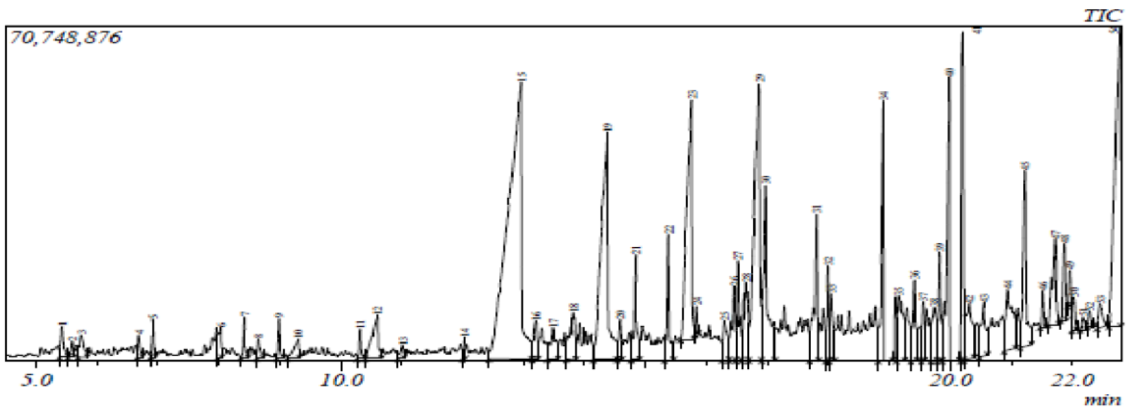

Figure 2: *GC-MS chromatogram of P. nitida stems bark*

Table 3: *Compounds Identified in GC-MS Study of P. nitida stem bark extract*

Peak No.	Retention Time (Rt)	Name of compound	Area Percentage %	Molecular mass	Molecular formular
1	5.432	Mesitylene	0.48	120	C_9H_{12}
2	5.593	Undecane	0.21	156	$C_{11}H_{24}$
3	5.738	2-benzoyl-8-octanelactam	0.49	245	$C_{15}H_{19}NO_2$
4	6.681	Nonanal	0.26	142	$C_9H_{18}O$
5	6.914	Dodecane	0.34	170	$C_{12}H_{26}$
6	8.030	Octanoic acid	0.44	144	$C_8H_{16}O_2$
7	8.416	Dodecane	0.37	170	$C_{12}H_{26}$
8	8.643	Octane, 1,1'-oxybis-	0.25	242	$C_{16}H_{34}O$
9	8.981	2-Tridecenal,(E)-	0.35	196	$C_{13}H_{24}O$
10	9.297	Nonanoic acid	0.45	158	$C_9H_{18}O_2$
11	10.313	2-Undecenal	0.28	168	$C_{11}H_{20}O$
12	10.600	n-decanoic acid	1.31	172	$C_{11}H_{20}O_2$
13	11.013	1,3,2,dioxaborolane,2-ethyl-4-(oxiranyl propyl)-	0.21	184	$C_9H_{17}BO_3$
14	12.032	2-methyltetracosane	0.21	352	$C_{25}H_{52}$
15	12.955	Dodecanoic acid	18.34	200	$C1_2H_{24}O_2$
16	13.200	6,9,12-Octadecatrienoic acid,phenylmethyl	0.67	368	$C_{25}H_{36}O_2$
17	13.47	Decan 1-chloroacetate,1,2,4a,5,8atetramethyl-1,2,3,4,4a7	0.89	176	$C_{10}H_{21}Cl$

18	13.816	Acetate, (2,4a,5,8a-tetramethyl1,2,3,4,4a,7,8,8a	1.18	250	$C_{16}H_{26}O_2$
19	14.371	Tetradecanoic acid	7.90	228	$C_{14}H_{28}0_2$
20	14.580	Octadecanoic acid, (2-phenyl-1,3dioxolan-4-yl)methyl ester, cis-	0.54	446	$C_{28}H_{46}0_4$
21	14.833	2-Pentadecanoic,6,10,14trimethyl-	1.37	268	$C_{18}H_{36}O$
22	15.372	Hexadecanoic acid, methylester	1.42	270	$C_{17}H_{34}O_2$
23	15.754	n-hexadecanoic acid	5.95	256	$C_{16}H_{32}O_2$
24	15.832	Hexadecanoic acid, ethyl ester	0.16	284	$C_{18}H_{36}0_2$
25	16.291	Octadecanoic acid	0.91	284	$C_{18}H_{36}O_2$
26	16.451	2(3H)-furanone,5dodecyldihydro-	1.16	254	$C_{16}H_{30}O_2$
27	16.512	9-Octandecanoic acid,methylester,(E)-	1.20	296	$C_{19}H_{36}O_2$
28	16.647	Phytol(2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,)	1.73	296	$C_{20}H_{40}O$
29	16.859	9-octadecanoic acid,(E)-	7.49	282	$C_{18}H_{34}O_2$
30	16.970	Octadecanoic acid	3.78	284	$C_{18}H_{36}O_2$
31	17.799	Octacosane	2.14	394	$C_{28}H_{58}$
32	17.995	4,8,12,16-Tetramethylheptadecan4-olide.	0.99	324	$C_{21}H_{40}O_2$
33	18.042	9-Octadecanoic, (z)-	1.01	266	$C_{18}H_{34}O$
34	18.900	Eiocosane	2.45	282	$C_{20}H_{42}$
35	19.734	Cyclopentadecanone, 2-hydroxyl-	1.81	240	$C_{15}H_{25}O_2$
36	19.407	2-Methylhexacosane	1.16	380	$C_{27}H_{56}$

37	19.554	4,8-Methanoazulen-9-ol,decahydro-2,2,4,8-tetramethyl-	0.71	222	$C_{15}H_{26}O$
38	19.735	Acetic acid,10-chloroddecylester	1.18	234	$C_{12}H_{23}ClO_2$
39	19.820	Azulene,1,2,3,5,6,7,8, 8aoctahydro-1,4din	1.34	204	$C_{15}H_{24}$
40	19.992	Tetrapentacontane	3.88	758	$C_{54}H_{110}$
41	20.192	2-Isopropenyl-4a,8-dimethyl1,2,3,4,4a,5,6	3.20	204	$C_{15}H_{24}$
42	20.316	9,10-Secocholesta-5,7,10(19)triene-1,3-diol	1.91 100.00	488	$C_{30}H_{52}O_3Si$

Fifteen compounds representing 100% of the total components identified, were found in the *P. nitida* leaf extract via the GC-MS result displayed in Figure 1. The chief compounds discovered were 3-O-methyl-d-gluccose (70.56%), phytol (5.67%), hexanoic acid, 2-ethyl hexyl ester (13.93%), Benzofuran, 2,3-dihydro (1.88%), 2-methoxy-vinylphenol (1.65%), and the minor component are Benzyl alcohol (0.65%), 3-(3,6-dimethyl-3,6-dihydro-2H-pyran-2-yl) (0.25%) as seen in Table 2. Hence, this shows that *P. nitida* leaves contain good pharmacological components. This is similar to report by (Aghedo *et al.* 2021). Also, the GC-MS analysis of *P. nitida* stem bark shows that forty two components are present with Dodecanoic acid being the major component with an area peak of 18.34% as shown in Table 3. The GC-MS result of both extract shows three similar bioactive component which are: 9-octadecanoic, methylester with area peak of 0.89% for the leaves and 1.20% for the steam bark, Phytol with an area peak of 5.67% for the leaves and 1.73% for stem bark and Hexadecanoic acid, methylester with an area peak of 0.66% for the leaves and 1.42% for the stem bark.

Hexanoic acid have the property of antioxidant and antimicrobial activities (Rosemeyer and Bodoprost, 2007, Kala *et al*., 2011), octadecanoic acid possesses anti-inflammatory, hypocholesterolemic and antriarthritic activities which was earlier reported by some researchers (Ponnamma and Manjunath, 2012; Rani *et al*., 2009; Uma *et al*., 2009). Benzyl alcohol is used as a general solvent for inks, waxes, shellacs, paint, lacquers and epoxy resin; it is also used as antibiotics and anti-inflammatory medication. Phytol and n-Hexadecaonic acid (Palmitic acid) are valuable in producing cosmetic products, soaps and industrial mold release agents (Iyasele *et al.,* 2022). Palmitic acid has a wide range of uses in the food industry; for instance, since it is inexpensive and readily available, it is used to increase the texture and mouth-feel of food which as been processed (convenience food)

(USSAOs, 2015). Because palmitic acid helps cells produce energy, medical and health authorities advise consuming a limited amount of it in the diet. Furthermore, lung secretions, some signaling molecules that control cellular activity, and cell membranes all depend on palmitic acid (WHO, 2020). Natural resources are a plentiful source of lauric acid, which the body uses as a source of direct energy after processing it as a carbohydrate. Also, Lauric acid in pair with Myristic acid, are saturated fatty acids used in assessing the average serum cholesterol levels in humans. Again, 9-octadecanoic acid (Stearic acid) is a well-known antiviral and anti-inflammatory agent (Iyasele *et al.,* 2022; Marie, 2015).

In-Vitro Antioxidant Study

The 1,1-diphenyl-2picrylhydrazyl (DPPH) radical is a stable radical that can easily undergo reduction in the presence of an antioxidant. Its maximum absorption occurs in the UV spectrum at 517 nm, or dark purple to light yellow color (Scridhar *et al.,* 2019). The level of discoloration reveals the antioxidant's capacity for scavenging (Jayaprakasha *et al.,* 2004). This reaction is now widely used in the evaluation of free radical-scavenging activity because it is simple and convenient. According to the mechanism by which antioxidants prevent lipid oxidation, DPPH free radical scavenging is produced, and this leads to the estimation of antioxidant activity and the determination of free-radical scavenging potential (Baliyan *et al.,* 2022). The concentration of ascorbic acid and the methanol extract of Picralima niitida leaves and stem bark both significantly affects their capacity to scavenge DPPH radicals. The reference standard, ascorbic acid, and the leaves' and stem bark's capacity to scavenge free radicals is fairly similar. As a result, as demonstrated in Table 4 and Figure 3, the extracts showed a noteworthy antioxidant property when compared to the standard (ascorbic acid).

Table 4: *Antioxidant ability of leaves and stem bark extract of P. nitida*

Concentration (μg/mL)	Standard	PL (%)	PS (%)
1	58.02	31.01	32.49
2	58.86	41.35	31.43
5	59.49	43.46	36.50
10	60.97	49.16	40.08
20	61.39	51.69	42.41
50	63.08	59.07	44.30
100	63.50	53.16	39.03
200	64.56	55.91	58.44

Standard = Ascorbic acid PL = *Picralima nitida* Leave PS = *Picralima nitida* stem bark

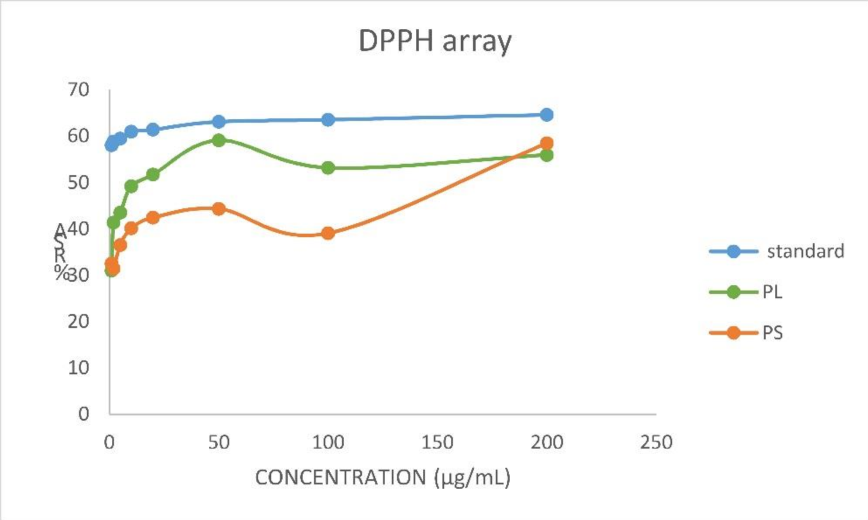

Figure 3: *Comparative antioxidant potential of leave and stem bark*

Plotting the percentage of DPPH scavenging activity against extract concentration allowed for the extrapolation of the plant extract concentration necessary to produce 50% inhibition (IC_{50}). Since IC_{50} values indicate the concentration that will inhibit 50% of the initial DPPH radical, they are inversely correlated with antioxidant activity (Ogbeide *et al.,* 2020a).

Table 5: *IC_{50} values of Leaf and stem bark extracts of P. nitidia and standard (ascorbic acid)*

Sample	IC_{50} (μg/mL)
Ascorbic acid	2.08 ± 1.280
Leaf extract	14.759±10.64
Stem bark	12.48±9.66

The leaves and stem bark's extracts were found to have IC_{50} values of 14.759±10.64 and 12.48±9.66 μg/mL respectively, while the reference value was 2.08 ± 1.280 μg/mL (Table 5. Because Uadia *et al*. (2023) noted that a compound's ability to scavenge free radicals increases with decreasing IC_{50}-value, hence, the result shows that the stem bark extract had stronger antioxidant activity compared to the leaf extract. The extracts' demonstrated antioxidant ability could be attributed to the previously identified phytochemicals (Uadia *et al.,* 2023; Annan *et al.,* 2013).

Antimalarial Study

Percentage (%) Parasitaemia of the Leaves and Stem bark Extract of Picralima nitida and Standard Drug (Chloroquine)

Table 6: *Effect of the leave extract and standard drug (chloroquine) on Plasmodium berghei NK65*

TREATMENT	DOSAGE (mg/kg)	D_5 (%±SEM)	D_6 (%±SEM)	D_7 (%±SEM)	D_8 (%±SEM)
No treatment	-	8.12±0.14	8.87±0.21	9.02±0.03	9.31±0.09
Extract	200	5.37±0.16	5.51±0.17	5.88±0.12	6.03±0.02
Extract	400	5.11±0.12	5.92±0.22	5.52±0.31	5.97±0.12
Extract	800	4.23±0.19	3.98±0.13	4.93±0.22	5.62±0.11
Chloroquine	5	0.24±0.08	0.27±0.12	0.31±0.07	0.36±0.02

No treatment = Negative control, Extract = Methanol extract of the of the leave of *Picralima nitida*, Chloroquine = positive control   SEM = Standard Error Mean

Table 7: *Effect of the stem bark extract and standard drug (chloroquine) on plasmodium berghei NK65*

TREATMENT	DOSAGE (mg/kg)	D_5 (%±SEM)	D_6 (%±SEM)	D_7 (%±SEM)	D_8 (%±SEM)
No treatment	-	8.12±0.14	8.87±0.21	9.02±0.03	9.31±0.09
Extract	200	4.88±0.21	5.11±0.09	5.63±0.16	5.64±0.18
Extract	400	3.81±0.11	4.62±0.13	4.96±0.24	5.41±0.13
Extract	800	3.39±0.16	3.98±0.19	4.41±0.12	4.76±0.06
Chloroquine	5	0.24±0.08	0.27±0.12	0.31±0.07	0.36±0.02

No treatment = Negative control, Extract = Methanol extract of the stem bark of *Picralima nitida*, Chloroquine = positive control   SEM = Standard Error Mean

Anti – Plasmodial Activity of the Leave and Stem Bark Extract of Picralima Niti

Table 8: *Percentage (%) Chemo-suppression of Plasmodium berghei berghei by the leave extract of Picralima nitida and standard drug (chloroquine)*

		Average % chemo-suppression			
Treatment	Dosage (mg/kg)	D_5 (%)	D_6 (%)	D_7 (%)	D_8 (%)
No treatment		-	-	-	-
Extract	200	33.87	37.88	34.81	35.23
Extract	400	37.07	33.26	38.80	35.88

Extract	800	47.91	55.13	45.34	39.63
Chloroquine	5	97.04	96.96	96.56	96.13

No treatment = Negative control, Extract = Methanol extract of the leave of *Picralima nitida*, Chloroquine = positive control SEM = Standard Error Mean

Table 9: *Percenatage (%) Chemo-suppression of Plasmodium berghei berghei by the Stem bark extract of Picralima nitida and standard drug (Chloroquine)*

Treatment	Dosage (mg/kg)	Average % chemo-suppression D5 (%)	D6 (%)	D7 (%)	D8 (%)
No treatment		-	-	-	-
Extract	200	39.90	42.39	37.58	39.42
Extract	400	53.08	47.91	45.96	41.89
Extract	800	58.25	55.13	51.11	48.87
Chloroquine	5	97.04	96.96	96.56	96.13

No treatment = Negative control, Extract = Methanol extract of the leave of *Picralima nitida*, Chloroquine = positive control SEM = Standard Error Mean

Table 10: *Average Chemo-suppression of the leave and stem bark extract*

Dosage (mg/kg)	(%) C.L	(%) C. SB
200	35.45±14.49	39.82±1.72
400	36.25±2.02	47.21±4.07
800	47.00±5.57	53.34±3.63

C.L=Chemo-suppression of the leave C.SB= Chemo-suppression of the stem bark

The data from antimalarial study shows that *Picralima nitida* has significant antimalarial activity. The extract's suppressive activity rose as the dosage was increased and given through D5 to D8, indicating that the extract's effects on the parasite's growth were dose-dependent. However, there was a reduction in the activity from D_5 to D_8 for both leaves and stem bark extract and the control; this is most likely caused by some of the juvenile parasites that developed into adults and were then released into the bloodstream after escaping the treatment. This is similar to the report by (Oghogho *et al.,* 2022; Ogbeide *et al.*, 2018; Jonville *et al.*, 2008). A comparative study of the chemo-suppression shows that the stem bark is more effective than the leave as shown in Figure 4. Although the bioactive components were not isolated, but the result shows that the extracts of *P. nitida* leave and stem bark possess anti-malarial bioactive components with great potentials.

4. CONCLUSION

A qualitative phytochemical analysis was conducted on water and ethyl acetate extracts of *Picralima nitida*. The preliminary phytochemical screening of the leaves and stem bark extracts of *P. nitida* was investigated and indicated the presence of alkaloids, saponins, terpenoids, tannins, reducing sugars, protein, phenolic compounds, glycosides, and carbohydrates; flavonoids was detected in the stem bark extract, distinguishing it from the leaves extract. The GC-MS of the leaves revealed 15 compounds while that of the stem bark extract showed the presence of 42 compounds containing good bioactive components. In the antioxidant studies, the stem bark extract demonstrated superior antioxidant activity compared to the leaves extract. Both the leaf and steam bark extracts of *Picralima nitida* demonstrated promising anti-plasmodial and anti-malarial activities; meanwhile, the stem bark extract was found to be more effective than the leaf extract. These findings are promising and warrant further investigation for the isolation, identification and characterization of the active compounds present in *P. nitida* for the development of the plant into novel drugs and potent medicinal agents that are relatively safe.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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