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In Vivo Antimalarial Potential of Methanol and Aqueous Extracts of *Newbouldia laevis* on *Plasmodium berghei-infected* (NK65) Infected Mice

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ABSTRACT

This research evaluated the *in vivo* antimalarial activity of methanol and aqueous extracts of *Newbouldia laevis* in *Plasmodium berghei*-infected (NK65) mice. The percentage yields of crude methanol and aqueous extracts from the plant's leaves, roots and stem bark were measured. Phytochemicals profiling was carried out on the methanol root extract using Gas Chromatography-Flame Ionization Detector (GC-FID). Antimalarial activity was evaluated using a standard *in vivo* 4-day curative assay in *P. berghei*-infected (NK 65). Root extract had aqueous yield (46.98%) and methanol extracted most from stem bark (22.42%). GC-FID revealed ribalinidine (62.19 µg/mL) was the most abundant, followed by saponin (13.17 µg/mL), sapogenin (12.73 µg/mL), rutin (10.84 µg/mL), kaempferol (9.45 µg/mL) and others detected in lower amounts. The antimalarial activity was evaluated by meas parasitemia, from day one to day 14. Methanol leaf (6.00±1.00%) and aqueous Bark (2.00±1.00%) presented the highest and least parasitaemia count at Day 7 respectively. At day 14, the plant extracts showed varying levels of inefficacy, with parasitaemia count for methanol leaf (45.00±0.00%) and methanol root (3.00±1.00%) extracts showing the highest and least parasite burden. These findings indicate that *N. laevis* methanol root extract as a potential alternative treatment for malaria and good source for drug development having shown potent antiplasmodial properties, emphasizing also the need for proper dose regulation, especially in resource-limited settings.

Keywords: Parasitemia, antiplasmodial, therapeutic, antioxidant, histopathology, phytochemicals

1. Introduction

Malaria is a deadly disease that has persisted as a global health burden for centuries, causing significant loss of life annually. The disease remains dynamic, evolving, and concentrated primarily in some of the world's poorest nations (Alonso, 2021). The World Health Organization (WHO) reports that the African region bears the highest malaria burden, contributing to economic setbacks and affecting mostly children under five years old. This high prevalence is primarily due to *Plasmodium falciparum*, the most virulent among the five human malaria parasite species (Oboh et al., 2018; WHO, 2022a). In 2021, approximately 247 million malaria cases were reported across 84 malaria-endemic countries worldwide, with Africa accounting for 234 million cases (95%). Nigeria recorded the highest number of malaria-related deaths, with an estimated 619,000 fatalities that year (WHO, 2022b).

The increasing resistance of malaria parasites to recommended drugs, including artemisinin-based compounds (Ariey et al., 2014; Birnbaum et al., 2020), coupled with the high-cost and widespread counterfeiting of antimalarial drugs, has made malaria treatment and control increasingly challenging (Imran et al., 2021). These challenges necessitate the urgent search for more potent and safer alternative therapeutic agents with novel mechanisms of action.

For centuries, humans have relied on medicinal plants to prevent and treat a wide range of diseases, including malaria (Yineger & Yewhalaw, 2007). The use of medicinal plants for treating various diseases has gained increasing attention from researchers, as it forms the basis of modern pharmacology and has led to the discovery of several plant-derived therapeutic agents (James et al., 2018). Despite the lack of comprehensive pharmacological validation and clinical trials, medicinal plants continue to be widely accepted as a primary healthcare source, especially in African nations like Nigeria (Okaiyeto & Oguntibeju, 2021; Asfaw et al., 2023; Matowa et al., 2020; Van Vuuren et al., 2022). It is estimated that approximately 80% of the global population depends on medicinal plants due to their affordability and accessibility (Li et al., 2020; Mlilo & Sibanda, 2022).

Nigeria has a rich heritage of folk medicine, where various herbal preparations using different plant parts serve as alternatives or complementary therapies to conventional medicine in preventing and treating numerous diseases (Mgbeahuruike et al., 2019; Balogun, 2021). Many Nigerians, particularly those in rural and peri-urban areas, rely on folk medicine due to its availability, the high cost of conventional medicine, poor healthcare infrastructure, and limited

access to allopathic medicine (Osuchukwu et al., 2017). Others prefer herbal formulations because they trust them, as these remedies have been traditionally used for generations.

Several studies have reported numerous medicinal plants used in malaria treatment in Nigeria, either individually or as polyherbal formulations (Adewole, 2020; Ajao et al., 2022). Many of these plants have demonstrated antimalarial properties (Evbuomwan et al., 2023a; Oluba, 2019; Adepiti et al., 2022) and could serve as potential leads for developing more potent antimalarial agents. This is particularly relevant given that two of the most successful antimalarial drugs—quinine and artemisinin—were derived from plants (Antoine et al., 2014; Gachelin et al., 2017). Thus, exploring Nigeria's rich biodiversity through ethnobotanical and pharmacological research is crucial.

Newbouldia laevis is a tropical plant and it is among the most useful plants in Africa, ever greenish plant with a characteristic shiny dark green leaves and large purple flowers (Bafor & Sanni, 2009). In Nigeria Igbos call it ogilisi or ògírìsì, Hausas call it Aduruku, and Yorubas call it Akoko. *Newbouldia laevis* bark and leaves can be used for treating arthritis and rheumatism. It works as a painkiller, laxative and treating diarrhea and dysentery (Egba et al., 2014).

The stem bark and root of *N. laevis* has antibacterial activities, can be used for treating swellings, and oedema arising through dietary deficiency. The leaf extract can be used for both eye and ear treatments and also as an antidote for treating venomous stings and bites (Egba et al*.,* 2014). However, there is limited literature on the antimalaria potentials of *N. laevis.* This is what informed this study.

2. Materials and Methods

2.1 Plant Collection, Preparation and Extraction

Plant samples of *Newbouldia laevis* were harvested in August 2015, from a farm in Umuezeala (Umudaranwaneri) village of Awo-Omamma in Oru-East Local Government Area of Imo State, Nigeria. Geographic coordinates of the sample site were obtained using the Mobile Topographer app, placing the primary sampling site at 5.66849354° N, 6.95121404° E (WGS 84), altitude 158.60 m. In UTM Zone 32N, this corresponded to Easting 273081.078 m, Northing 626960.199 m, height 139.17 m above MSL. Plant samples were identified by a taxonomist, Dr. Francis Iwu of the Department of Forestry and Wildlife Technology, FUTO. A voucher specimen (FHI 29271) was deposited at Ujor Forestry Harvester Herbarium, Ibadan,

and another (FUTO/H100125) at FUTO's Department of Forestry and Wildlife Technology Herbarium.

The method of Buss and Butler (2010) was adopted for the plant preparation and extraction. The plant parts including; roots, stem barks, and leaves were harvested in large quantities, washed thoroughly in tap water. The root, leaves and stem bark were cut into pieces, and dried separately in the laboratory at room temperature (25± 3^0C) for about 7 weeks before pulverizing into powdered form using crusher machine. Using crude method, 100g each of the pulverized parts were macerated separately in deionized water and methanol, for 48 h. Each sample was filtered using Whatman number 1 filter paper (this was to get rid of residues). All aqueous and methanol filtrates (infusions) were concentrated using rotary evaporator. The extracts were stored in the refrigerator at 4^OC until required. Also, the percentage yield of each extract was determined.

2.2 Animals and animal husbandry

The protocol for the use of Mice in this research got an approval from the research ethics committee resident in the Biochemistry Department of the Federal University of Technology, Owerri, Nigeria with approval number: FUTO/SOSC/BCH/2015/B012. The research was carried out from August to December 2015. Thirty (30) adult Swiss albino mice (male) weighing 24±2 g were procured from the Department of Veterinary Parasitology of University of Nigeria Nsukka (UNN). The animals were transported to animal house of Department Biochemistry FUTO, allowed free access to standard mice feed (Vital starter) and water and were acclimatized for two weeks.

2.3 *In vivo* culture of the *Plasmodium berghei* (NK 65) in albino mice

The methods of David *et al*. (2004) and Peter and Anatoli (1998) were adopted for the *in vivo* culture of *Plasmodium berghei* (NK 65) in the animals. This was achieved by administering intraperitoneally the *Plasmodium berghei* (NK 65) infected red blood cells of mice into healthy mice as infected blood diluted with phosphate buffered saline (PBS) of pH 7.2ml, such that each 0.2ml contains approximately 10 x 10^7 infected red cells (indicating parasite per kg of body weight. Twenty four (24) hours after passaging the mice, parasitemia was confirmed in the test animals by preparing blood smears from tail vein blood of the passaged test animals. Giemsa stain was applied to the blood smears and viewed in a microscope at x100 objective (emersion oil). Afterwards, the infected and uninfected animals were allowed *ad libitum* standard laboratory mice feed (Vital starter) and water under standard laboratory conditions.

2.4 *In vivo* treatment of *Plasmodium berghei* (NK 65) infected albino mice

The *in vivo* treatment of *Plasmodium berghei* (NK 65) infected albino mice was performed using a 4-day curative standard test of Peter and Anatoli. (1998) as modified by David et al. (2004) and employing *Plasmodium berghei* (NK 65) rodent malaria parasite. The infected mice (30) were randomized into 10 groups of three mice per group. Forty-eight hours (48 hours) after infection with the *Plasmodium berghei* (NK 65), the plant extracts were administered to the experimental groups (group one to group six) at a dose of 100mg/kg body weight (bw) daily for four days (Table 1). The standard control drugs (artesunate and artemether) were administered to different groups based on their average body weight. Chloroquine (CQ) was also administered to the CQ standard control group at the standard dose of 10mg/kg body weight for four days. The mice in negative control group were not administered any extract or drugs (Untreated control). All extracts and standard drugs were intraperitoneally administered to the mice. The extracts were dissolved to the indicated suitable dose level in solution and suspension, the later requiring complete dissolution in 3%v/v Tween 80. Daily treatments were administered for 4 consecutive days which started 48 h after infection, receiving an aggregate of 4 intraperitoneal doses (David et al., 2004; Ene *et al*., 2015). Afterwards, blood smears were prepared from tail vein blood samples collected from each mouse in the group, fixed in methanol, and subsequently stained with Giemsa at pH 7.2 and examined under the microscope using x100 (under immersion oil) to assess the parasitaemia level. The percentage parasitaemia was determined using the technique described by Iwalewa et al. (1997) as:

$$\text{Percentage parasitaemia} = \frac{\text{Number of parasitaemia in treated}}{\text{Number of parasitaemia in control}} \times \frac{100}{1}$$

This is always assumed to be:

$$\text{Percentage parasitaemia} = \frac{\text{Number of parasitaemia in treated}}{500} \times \frac{100}{1}$$

Table 1 *In vivo* Parasitemia Studies using Infected Albino Mice

Groups	Group Identity	Treatment
Group 1	Aqueous Leaf Extract	100 mg/kg of Aqueous Leaf Extract
Group 2	Methanol Leaf Extract	100 mg/kg of Methanol Leaf Extract
Group 3	Aqueous Root Extract	100 mg/kg of Aqueous Root Extract

Group 4	Methanol Root Extract	100 mg/kg of Methanol Root Extract
Group 5	Aqueous Bark Extract	100 mg/kg of Aqueous Bark Extract
Group 6	Methanol Bark Extract	100 mg/kg of Methanol Bark Extract
Group 7	Artemether	1.6 mg/kg of Artemether (Standard Drug)
Group 8	Artesunate	1.6 mg/kg of Artesunate (Standard Drug)
Group 9	Chloroquine	10 mg/kg of Chloroquine (Standard Drug)
Group 10	Negative Control	3% v/v Tween 80 (No extract/drug)

2.5 Phytochemical Evaluation of Methanol Root Extract of *N. laevis* Plant using Gas Chromatography-Flame Ionization Detector (GC-FID)

The phytochemical evaluation of part of *N. Laevis* plant with the highest antimalaria activity was analyzed by gas chromatography, following the method described by Kelly and Nelson (2014). One gram (1 g) of the methanol root extract was weighed into a test tube, followed by the addition of 15 mL ethanol and 10% of 50%w/v potassium hydroxide. The mixture was incubated in a water bath for 60 mins to allow the reaction to proceed. After the reaction, the product was transferred to separation funnel. The test tube was rinsed sequentially with 20 mL ethanol, 10 mL hot water and 3 mL hexane and the washings were combined in the funnel. The solution was then dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was dissolved in 1000 µL pyridine, and a 200 µL aliquot was transferred to a vial for analysis

Phytochemical quantification of the methanol root extract of *N. laevis* plant was carried out by the method of Bezerra and Filho. (2014) using a BUCK M910 GC-FID. The BUCK M910 is equipped with a RESTEK 15 m MXT-1 column (15 m × 250 µm × 0.15 µm). The injector temperature was maintained at 280 °C, with splitless injection of 2 µL of methanol root extract of *N. laevis* (sample) at a linear velocity of 30 cm s^{-1}. Helium (5.0 Pa s) served as the carrier gas at a flow rate of 40 mL min^{-1}. The oven was initially set at 200 °C, then programmed to increase to 300 °C. Phytochemicals were quantified based on the ratio of the area-to-mass of the internal standard to the area of each identified compound and concentration were expressed as µg/g

2.6 Statistical Analysis

All the data were subjected to analysis of variance (ANOVA) using SPSS software (version 20.0 SPSS Inc., Chicago, IL, USA). Results are presented as mean ± standard deviation (SD), and differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Percentage Yield of Crude Extracts of Various Plant Parts of *N. laevis* Using Aqueous and Methanol Solvents

The results presented in Table 2 show the percentage yield of crude extracts of various plant parts of *N. laevis* using aqueous and methanol solvents, respectively. The aqueous extraction used 800 ml of deionized water on 100 g of the sample. The leaf sample produced 34.16 g of crude extract, resulting in a 34.16% yield, root yielded the highest extract with 46.98 g (46.98%) and stem bark produced 40.74 g (40.74%). The results show that the root extract presented the highest extraction efficiency with water, followed by stem bark, and then leaf. In methanol extraction, 600 mL of methanol was used on 100 g samples. The leaf samples produced 12.77 g of extract, which gave a yield of (12.77%), root produced 17.84 g (17.84%) and the stem bark yielded the highest methanol extract with 22.42 g (22.42%). The extraction using methanol showed that the stem bark yielded the most extract, followed by the root, then the leaves suggesting that the stem bark has more methanol-soluble (possibly less polar) phytochemicals compared to the root and leaf.

Table 2: Percentage Yield of Aqueous and Methanol Crude Extracts of Various Plant Parts of *N. laevis*

Samples	Extraction Solvent		Sample weight (g)	Weight of extract (g)	Yield of extract (%)
	Deionized H_2O (ml)	Methanol (ml)			
Leaf	800		100	34.16	34.16
Root	800		100	46.98	46.98
Stem Bark	800		100	40.74	40.74
Leaf		600	100	12.77	12.77
Root		600	100	17.84	17.84
Stem Bark		600	100	22.42	22.42

3.2 Gas Chromatography-Flame Ionization Detection Studies

The quantitative phytochemical screening of methanol root extract of *N. laevis* is presented in Table 3. The results of GC-FID analysis present a diverse phytochemicals with varying

concentrations (Table 3). Among the detected compounds, ribalinidine presented the highest concentration measuring 62.19 µg/mL. Saponin (13.17 µg/mL), sapogenin (12.73 µg/mL), rutin (10.84 µg/mL), kaempferol (9.45µg/mL), tannin (7.16 µg/mL) and catechin (5.52 µg/mL) showed moderate concentration. respectively. Phenol (3.57 µg/mL), lunamarine (2.0076 µg/mL), epicatechin (1.76 µg/mL) and anthocyanin (1.25µg/mL) recorded low concentrations. Anti-nutrients such as oxalate (4.56 µg/mL) and phytate (0.24 µg/ml) were recorded. Spartein (0.0002 µg/ml) presented the least amount of detected phytochemicals.

Table 3 GC-FID Values of Phytochemical Constituents of Methanol Root Extract of *N. laevis*

Compounds	Class	Retention Time	Area	Height	Concentration (µg/ml)
Spartein	Alkaloid	0.08	267.22	139.932	0.0002
Anthocyanin	Flavonoid	2.39	12509.64	170.461	1.2506
Oxalate	Anti-nutrient	4.113	6067.48	88.97	4.5578
Tannin	Polyphenol	6.02	18913.54	250.061	7.1554
Rutin	Flavonoid	10.366	18590.14	264.24	10.8438
Phenol	Simple Phenolic	12.966	5269.61	79.557	3.565
Epicatechin	Flavonoid	17.963	10410.47	150.236	1.7627
Lunamarine	Alkaloid	20.313	12054.11	170.475	2.0076
Saponin	Saponin	22.726	9023.33	128.483	13.1672
Sapogenin	Saponin derivative	27.533	11294.29	156.987	12.729
Ribalinidine	Alkaloid	32.996	14513.32	196.783	62.19
Phytate	Anti-nutrient	34.583	5595.26	83.414	0.2372
Kaempferol	Flavonoid	39.196	10084.17	140.192	9.4519
Catechin	Flavonoid	44.166	10566.65	145.175	5.5158

3.3 Parasitaemia Count

The result of the percentage parasitaemia is presented in Tables 4 and 4b showing results of parasitemia count of day 0 to day 14. No significant variation was observed in the percentage parasitaemia count across the different treatment groups on Day Zero (Day 0). The lowest parasitaemia count was presented in the Methanol Bark extract group (4.00±0.00%), while the highest was recorded in the Aqueous Bark extract group (5.67±2.08%). Other groups expressed comparable values, indicating uniform baseline parasitaemia levels before treatment commenced. A slight increase in parasitaemia was observed across most groups on Day 1. The

highest parasitaemia count was in the Methanol Bark extract group (7.67±4.93%), the lowest was in the Artesunate group (2.33±0.57%) while the untreated control group showed increase (4.67±2.08%) in parasitaemia count, indicating progressive infection.

At Day 2, the Artesunate (1.33±0.58%) and Artemether (1.00±0.00%) groups presented the lowest parasitaemia count. The untreated control group (6.00±1.00%) and the Aqueous Bark extract group (3.33±2.31%) still maintained higher values of parasitaemia count, showing limited early efficacy of the plant extracts. At Day 3, the untreated group expressed continued parasitaemia count increase (8.00±2.00%), whereas the Methanol Root (1.33±0.58%) and Aqueous Bark (1.33±0.58%) extracts presented the lowest parasitaemia count. However, the artesunate group had a slight increase (2.33±1.53%) in parasitaemia count but remained lower than most plant extract groups. At Day 4, most of the treatment groups maintained low parasitaemia count, with Methanol Root (2.00±0.00%), Aqueous Bark (2.00±1.00%) extracts, and Artesunate (1.00±0.00%) showing effective parasite suppression. The untreated control group continued to show significant rise (10.33±4.51%) in parasitaemia count, indicating unmitigated infection progression. At Day 5 to Day 7, the untreated control group presented a markedly higher parasitaemia count increasing from 11.33±6.81% to 15.33±8.39%. However, the artemether and artesunate groups showed the lowest parasitaemia counts fluctuating from 0.33±0.58 to 1.33±1.53% and 1.00±0.00 to 1.00±0.00% respectively. Whereas the Methanol leaf (6.00±1.00%) and at and Aqueous Bark (2.00±1.00%) presented the highest and least parasitaemia count at Day 7.

Furthermore, at Day 8, a drastic rise in parasitaemia count was observed in the untreated group (15.67±6.43%), while artesunate-treated mice had the lowest parasitaemia count (0.33±0.58%). Methanol Root extract (3.33±1.53%) and Aqueous Root extract (2.67±0.58) presented continued moderate effectiveness in suppression of parasitaemia. At Day 9, the percentage parasitaemia count in the untreated group sharply increased (29.33±9.29%), showing disease aggravation. While Some plant extracts including Methanol Root (3.67±0.58) extract continued to maintain relatively low parasitaemia count at day 9. However, the artesunate and artemether groups remained effective with nearly zero parasitemia (0.67±0.58% and 0.33±0.58%) respectively.

Similarly, at Day 10, a drastic increase in parasitaemia count was recorded in the untreated group (36.67±7.64%), while among the plant extracts, Methanol Root (5.33±0.58%) showed the least parasitaemia count, while Aqueous Leaf extract (18.33±0.58%) presented a

significantly increased parasitaemia count. At Day 11 to 14 the untreated group presented continued increase in parasitemia count and peaked at 50.00±1.00%. Artesunate and artemether groups maintained nearly zero parasitaemia count with minimal parasitaemia count ≤0.67%. The plant extracts at this period showed varying levels of inefficacy, with some groups showing steady increases in parasitaemia count and Methanol Leaf with a parasitaemia count of 45.00±0.00 presented the highest parasite burden. However, the Methanol Root extract with a parasitaemia count of 3.00±1.00% presented the lowest parasitaemia among plant extracts.

Table 4 Parasitemia count of day 0 - day 7

Plant parts extracts and standard drug	Number of Animals	Treatment Dosage (mg/kg)	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Aqueous Leaf	3	100	5.60±1.53^{a}	6.67±3.51^{a}	2.33±0.58^{ab}	2.33±0.58^{ab}	2.00±0.^{00abc}	3.00±1.73^{a}	3.33±0.5^{8}	3.00±0.00^{ab}
Methanol Leaf	3	100	4.33±0.58^{a}	4.33±2.08^{a}	3.67±2.08^{b}	2.67±1.15^{ab}	5.00±0.00^{bc}	2.67±0.58^{a}	4.33±0.5^{8}	6.00±1.00^{ab}
Aqueous Root	3	100	5.67±0.58^{a}	5.00±0.00^{a}	2.67±0.58^{ab}	3.33±1.53^{ab}	4.00±0.58^{abc}	4.00±2.00^{a}	6.00±1.00^{bc}	4.33±0.58^{ab}
Methanol Root	3	100	4.67±1.55^{a}	7.33±3.79^{a}	2.33±1.53^{ab}	1.33±0.58^{a}	2.00±0.00^{abc}	1.67±0.58^{a}	4.00±0.00	5.33±0.058^{ab}
Aqueous Bark	3	100	5.67±2.08^{a}	5.33±4.16^{a}	3.33±2.31^{ab}	1.33±0.58^{a}	2.00±1.00^{abc}	2.00±1.00^{a}	3.67±0.5^{8}	2.67±0.58^{a}
Methanol Bark	3	100	4.00±0.00^{a}	7.67±4.93^{a}	2.67±0.58^{ab}	2.33±0.58ab	1.67±0.58^{ab}	3.00±1.00^{a}	6.33±1.53^{c}	4.00±0.00^{ab}
Artemether	3	1.6	4.00±1.53^{a}	3.67±2.08^{a}	1.00±0.00^{a}	1.67±0.58^{a}	2.00±1.00^{abc}	0.33±0.58^{a}	0.67±0.58^{a}	1.33±1.53^{a}
Artesunate	3	1.6	5.00±1.73^{a}	2.33±0.57^{a}	1.33±0.58^{ab}	2.33±1.53a^{b}	1.00±0.00^{a}	1.00±0.00^{a}	1.67±0.58^{ab}	1.00±0.00^{a}
Chloroquine	3	10	4.67±0.58^{a}	6.33±1.53^{a}	3.00±1.00^{ab}	4.67±2.08^{b}	5.67±3.78^{c}	5.33±4.04^{a}	7.67±3.79^{c}	9.00±5.29^{b}
Untreated	3	Tween 80	5.00±1.73^{a}	4.67±2.08^{a}	6.00±1.00^{c}	8.00±2.00^{c}	10.33±4.51^{d}	11.33±6.81^{b}	13.00±6.08^{d}	15.33±8.39^{c}

Values are presented Mean ± Standard Deviation of triplicate determinations. Columns bearing different superscript alphabets indicate significant difference at $p < 0.05$.

Table 4b Parasitemia count of day 8 to day 14

Plant parts extracts and standard drug	Number of Animals	Treatment Dosage (mg/kg)	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
Aqueous Leaf	3	100	3.67±2.08^{a}	14.33±4.51^{c}	18.33±0.58^{e}	19.67±0.5^{8e}	26.00±4.00^{d}	36.00±0.00^{d}	40.00±0.00^{e}
Methanol Leaf	3	100	3.67±0.58^{a}	5.67±0.58^{ab}	13.67±3.51^{de}	15.00±7.81^{cde}	25.67±6.66^{d}	36.00±2.65	45.00±0.00^{f}
Aqueous Root	3	100	2.67±0.58^{a}	6.00±0.00^{ab}	6.00±0.00^{abc}	17.33±2.51^{de}	24.33±4.93^{d}	33.00±1.73^{d}	41.00±1.00^{ef}
Methanol Root	3	100	3.33±1.53^{a}	3.67±0.58^{ab}	5.33±0.58^{abc}	5.33±1.53^{ab}	5.00±1.00a^{b}	4.33±0.58^{a}	3.00±1.00^{a}
Aqueous Bark	3	100	6.00±4.36^{ab}	6.33±0.58^{ab}	7.67±1.53^{cde}	9.00±4.58b^{c}	11.33±4.04b^{c}	21.00±4.58^{c}	24.00±7.81^{c}
Methanol Bark	3	100	4.00±2.00^{ab}	4.00±2.00^{ab}	4.33±0.58^{ab}	5.00±3.00^{ab}	15.00±7.00^{c}	24.33±4.51^{c}	32.33±2.52^{d}
Artemether	3	1.6	1.00±1.00^{a}	0.33±0.58^{a}	0.00±0.00^{a}	0.33±0.58^{a}	0.67±1.15^{a}	0.67±1.15^{a}	0.67±0.58^{a}
Artesunate	3	1.6	0.33±0.58^{a}	0.67±0.58^{a}	0.67±0.58a	1.00±1.00^{a}	1.00±1.000^{a}	0.67±0.58^{a}	0.67±0.58^{a}
Chloroquine	3	10	9.33±4.16^{b}	10.33±4.93^{bc}	11.00±6.25^{cd}	11.33±6.43b^{cd}	11.67±6.66^{bc}	11.67±6.66^{b}	15.67±1.52^{b}
Untreated	3	Normal saline	15.67±6.43^{c}	29.33±9.29^{d}	36.67±7.64^{f}	41.00±5.57^{f}	43.67±7.77^{e}	47.33±0.58^{e}	50.00±1.00^{g}

Values are presented Mean ± Standard Deviation of triplicate determinations. Columns bearing different superscript alphabets are significant at $p < 0.05$.

4. Discussion

The aqueous extraction with 800 ml of deionized water on 100 g of the powdered plant material presented the highest yield from the root (46.98%), followed by the stem bark (40.74%) and the leaf (34.16%), thus indicating the root contains greater quantity of water-soluble compounds relative to the other parts. Also, methanol yielded 22.42% from stem bark, root (17.84%) and leaf (12.77%). These results are consistent with earlier research which indicated varying extraction efficiencies based on solvent polarity and plant part. Ugwu et al. (2019) reported methanol extracts of *N. laevis* root bark and leaf exhibited different phytochemical profiles and antibacterial activities, highlighting the importance of solvent selection in phytochemical studies.

The quantitative phytochemical analyses of the methanol root extract of *Newbouldia laevis* revealed a rich array of compounds widely recognized for their antioxidant and antimalarial activities. Tannins and flavonoids, such as rutin and kaempferol, are potent antioxidants. Their mode of action involves scavenging free radicals, chelating metal ion, and suppresssing oxidative enzyme activity (Othman et al., 2019; Jain et al., 2024). Their chemical structures allow hydrogen donation, thereby facilitating the neutralization of reactive oxygen species (ROS) (Yokozawa et al., 1998). The appreciable amount of the flavonoids in the methanol root extract *N. laevis* as potent antioxidants may also express protective effects against parasite-induced oxidative stress in the infected mice (Ene et al 2014; Ujowundu et al., 2010). Rutin and kaempferol have also demonstrated *in vitro* antiplasmodial activity against *Plasmodium falciparum* (Al-Huqail, et al., 2023). This can be ascribed to the methanol root extract's effectiveness reducing parasitaemia level. Whereas Tannins though have anti-nutritional properties also possess antimicrobial and antiparasitic activities, while catechin is a strong antioxidant and anti-inflammatory agent.

Saponins and sapogenins, which showed substantial quantities, can modulate oxidative stress and exhibit immunostimulatory and antiparasitic activities due to their ability to disrupt cell membranes. Studies indicate that saponin and sapogenin possess anti-parasitic, immunomodulatory, and anti-inflammatory properties, which could play a role in the therapeutic effects observed in parasitized animals (Huang et al 2021; Zhang et al., 2020 ; Liu et al 2024).

Alkaloids, including trace amounts of spartein, demonstrates diverse pharmacological effects, with excellent prove of antimalarial efficacy, with quinine being a classical example (Abou et al., 2019; Uzor, 2020). Lunamarine and Epicatechin identified at appreciable concentrations and are known to express antioxidant and antiparasitic potential. Anthocyanin can also contribute to the antioxidant profile of the methanol root extract *N. laevis*.

Ribalinidine, which showed the highest abundance of 62.19 µg/ml, is less studied for antimalarial activity, but its prominence suggests a possible bioactive role. Also, the amount of saponin in methanol root extract of *N. laevis* may have significant role in parasitaemia clearance. Saponins may be implicated in antiplasmodial activity through membrane disruption of the parasite, and phenolic compounds may enhance these effects synergistically. Overall, the presence of diverse phytochemicals in methanol root extract of *N. laevis* supports the combined antioxidant and antimalarial activities recorded and equally highlights the therapeutic potential of the plant extract.

From Day 8 onward, parasitemia levels in the untreated group escalated significantly (15.67±6.43), indicating rapid malaria progression without therapeutic intervention. This trend continued, with parasitemia reaching 29.33±9.29 on Day 9 and 36.67±7.64 by Day 10, highlighting the urgent necessity for effective treatment strategies. In contrast, standard antimalarial drugs demonstrated superior efficacy. Both Artemether and Artesunate consistently maintained low parasitemia levels (0.33±0.58) from Day 8 through Day 10, reflecting their potent antimalarial properties. This is consistent with findings from Ebenebe et al. (2018), who reported high efficacy of artemisinin-based combination therapies in Nigerian children (Derebe et al., 2021).

Among the plant extracts tested, the methanol root extract exhibited the most promising antimalarial activity. On Day 8, it achieved a parasitemia level of 3.33±1.53, slightly increasing to 3.67±0.58 on Day 9 and 5.33±0.58 by Day 10. These findings indicate a moderate level of efficacy in suppressing plasmodium proliferation. Comparable results were reported in studies evaluating methanolic root extracts of *Dorstenia barnimiana* and different plants, which demonstrated significant parasitemia suppression in *Plasmodium berghei*-infected mice (Kifle et al., 2020; Derebe et al., 2021; Ayalew & Bekele, 2021).

The Aqueous Root extract likewise demonstrated moderate effectiveness, with parasitemia levels of 2.67±0.58 on Day 8. However, its efficacy diminished over time, indicating a potential

need for optimization or combination with other treatments. This observation aligns with findings from studies on Terminalia brownii, where methanolic extracts exhibited higher antimalarial activity compared to aqueous extracts (Biruk et al., 2020)

By Day 10, parasitemia in the untreated group peaked at 36.67 ± 7.64, confirming severe disease progression. Standard antimalarial drugs, Artemether and Artesunate, maintained low parasite levels (≤ 1.00), underscoring their efficacy. Among plant-based treatments, the Methanol Root extract of *N. laevis* demonstrated the most significant antimalarial activity (5.33 ± 0.58), while the aqueous leaf extract showed diminished efficacy (18.33 ± 0.58). On Day 14 the Methanol Root extract demonstrated superior performance (3.00 ± 1.00), consistent with studies highlighting the antimalarial potential of methanolic root extracts from various medicinal plants (Enenebeaku et al., 2021). In contrast, both Aqueous Leaf (40.00 ± 0.00) and Methanol Leaf (45.00 ± 0.00) extracts were ineffective over the long term, while bark extracts showed moderate but unsustained efficacy.

Comparatively, Artemether and Artesunate rapidly cleared parasites and sustained low parasitemia throughout the study. However, emerging artemisinin resistance in parts of Africa is a growing concern. Mutations in the Plasmodium falciparum kelch13 gene, such as R561H in Rwanda and A675V and C469Y in Uganda, have been linked to delayed parasite clearance, indicating partial resistance to artemisinin-based therapies (Balikagala et., 2021: Assefa et al., 2024).

Chloroquine exhibited limited efficacy, with a parasitemia count of 15.67 ± 1.52 on Day 14, suggesting partial resistance. This aligns with historical data indicating widespread chloroquine resistance in *P. falciparum* strains, leading to its replacement by artemisinin-based combination therapies (ACTs) in many regions (WHO, 2022).

The biochemical implications of the observed antimalarial activity of *N. laevis* methanol root extract are closely tied to its rich phytochemical composition. The methanol root extract consistently demonstrated significant suppression of parasitemia from Day 8 to Day 14, highlighting its potent antiplasmodial properties. This sustained efficacy points to the involvement of bioactive compounds in the root extract, which are likely attributable to its significant antimalarial activity.

A key group of compounds in the methanol root extract are alkaloids, particularly sparteine and ribalinidine. Sparteine, an alkaloid commonly used in traditional medicine, is known to

interfere with the metabolism of plasmodium parasites. Its antimalarial mechanism is thought to involve inhibition of DNA replication and protein synthesis within the parasite. Sparteine also plays a role in blocking the critical process of heme detoxification, which is essential for the survival of Plasmodium species. This disruption increases oxidative stress within the parasite, ultimately leading to its death. Ribalinidine, another alkaloid present in the extract, is a quinoline-type compound that resembles classical antimalarial drugs like chloroquine. It is believed to interfere with nucleic acid synthesis in the parasite, further contributing to its antiplasmodial activity. Lunamarine, also found in the extract, shares similar properties, adding to the overall efficacy of the extract against malaria (Kinghorn & Balandrin, 2014; Enenebeaku et al., 2022).

In addition to alkaloids, the extract contains various flavonoids, such as kaempferol, rutin, catechin, and epicatechin. These flavonoids exhibit antioxidant and immune-modulatory properties, which contribute to their antimalarial effects. By disrupting mitochondrial function and interfering with nucleic acid synthesis, flavonoids can inhibit parasite growth. Specifically, kaempferol and rutin have been reported to impair the integrity of *Plasmodium falciparum* cell membranes and interfere with protein synthesis, ultimately leading to the suppression of the viability of the parasite (Lee et al., 2013; Taylor, 2015; Ene et al., 2016).

The methanol root extract also contains significant amounts of phenols and tannins, both of which possess well-documented antioxidant properties. These compounds contribute to mitigate the oxidative stress induced by malaria infection, which can damage host tissues and promote parasite growth. Additionally, phenols and tannins may directly affect parasites by denaturing proteins or inhibiting enzymes critical to parasite metabolism (Karou et al., 2018).

Saponins and sapogenins, which are well-known for their triterpenoid glycoside properties, also contribute to the antimalarial activity of the extract. These compounds have been shown to disrupt the membranes of plasmodium cells, leading to hemolysis and cytotoxicity. Furthermore, saponins and sapogenins can enhance the host's immune response, helping to clear the infection more effectively (Nafiu et al., 2021; Ujowundu et al., 2022).

The presence of lunamarine and ribalinidine, both quinoline-type alkaloids, further strengthens the extract's antimalarial potential. These compounds act by interfering with parasite DNA, disrupting replication and transcription processes that are essential for the parasite's survival (Kinghorn & Balandrin, 2014; Ujowundu et al., 2017; Enenebeaku et al., 2022).

The methanol root extract also presented other compounds, including anthocyanins, oxalates, and hydrogen cyanide (HCN). Anthocyanins are primarily recognized for their antioxidant effects and may help support the immune system during malaria infection. Although oxalates and HCN can be toxic at high concentrations, in trace amounts, they may exhibit antimicrobial or antiparasitic effects. However, their inclusion in therapeutic formulations would require careful dosage regulation and detoxification to ensure safety (Nyamai et al., 2016).The significant antimalarial activity exhibited by the methanol root extract of *N. laevis* is the result of synergistic actions of multiple phytochemicals, especially alkaloids, flavonoids, tannins, and saponins. These compounds collectively interfere with parasite metabolism, oxidative stress regulation, and structural integrity. The promising efficacy of this extract justifies further biochemical studies to isolate, characterize, and potentially formulate these active compounds into safe, effective antimalarial therapies.

5. Conclusion

This study investigated the *in vivo* antimalarial effects of methanol and aqueous extracts of *Newbouldia laevis* on *Plasmodium berghei*-infected mice (NK65). At Day 14 the methanol root extract significantly reduced parasitemia count comparable to standard drugs demonstrating strong antiplasmodial activity. The significant antimalarial activity exhibited by the methanol root extract of *N. laevis* was attributed to the synergistic actions of multiple phytochemicals, especially alkaloids, flavonoids, tannins, and saponins. These compounds collectively interfere with parasite metabolism, oxidative stress regulation, and structural integrity. The promising efficacy of this extract justifies further biochemical studies to isolate, characterize, and potentially formulate these active compounds into safe, effective antimalarial therapies.

Conflict of interest

All authors have declared that no conflict of interest exists.

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