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Antioxidant and antimalarial properties of hot aqueous leaf extracts of *Setaria megaphylla*, *Ageratum conyzoides* and *Chromolaena odorata*

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Abstract

This study investigated the antioxidant and antimalarial properties of leaf extracts of *Setaria megaphylla*, *Ageratum conyzoides* Linn, and *Chromolaena odorata* Linn, plants used in folkloric treatment of malaria. The plants were assessed for phenolic profile, antioxidant capacity and radical scavenging activities using standard methods. Single, binary and ternary combinations of the processed leaves were subjected to hot aqueous extraction, and evaluated for *in vivo* antiplasmodial efficacy against *Plasmodium berghei* NK 65 using Swiss albino mice. The plants' total phenolic profile varied as *S. megaphylla* (*Sm*; 118.06 µg/ml) > *C. odorata* (*Co*; 116.58 µg/ml) > *A. conyzoides* (*Ac*; 73.01 µg/ml), with catechin, dihydrocytisine and tannin being the most abundant phenolics. Total antioxidant capacities (TAC) and reducing power potentials (RPP) of the plants increased with increase in extract concentrations with *Sm* showing highest TAC (1.49 ± 0.02 mgAAE/g) and RPP (0.47 ± 0.01 mgAAE/g) values. A similar trend was observed for the hydroxyl, nitric oxide and DPPH radicals scavenging potentials, showing dose-dependent increases in scavenging potentials but with no observed significant ($p<0.05$) differences in activities at the highest extract dose of 400 µg/ml. The *in vivo* antimalarial study demonstrated that the combination of *A. conyzoides* and *S. megaphylla* was the most effective, significantly reducing parasitemia without causing mortality in the mice. This research highlights the potential of *S. megaphylla*, *A. conyzoides*, and *C. odorata,* especially the combination of their extracts as source of effective anti-malarial agents, and further confirms the folkloric use of hot aqueous extracts of the plants in malaria treatment.

Keywords: Phenolic profile; Total antioxidant capacity; Hydroxyl radical; Nitric oxide; DPPH

1. Introduction

Malaria remains a critical public health concern, particularly in tropical and subtropical regions of the world, affecting over 240 million people and causing approximately 600,000 deaths annually, with the majority occurring among children in sub-Saharan Africa (WHO, 2023). Countries such as Nigeria, the Democratic Republic of Congo, and Kenya bear the greatest burden (WHO, 2022). Malaria is caused by Plasmodium parasites, with *Plasmodium falciparum* being the most lethal and responsible for the highest number of malaria-related deaths (Okeke et al., 2023). In Nigeria, malaria is endemic, with about 97% of the population at risk, leading to significant economic and healthcare burdens (NMEP, 2023).

Traditional medicine continues to play a key role in malaria treatment, particularly in resource-limited settings where conventional medicines may be expensive or inaccessible (Adamu et al., 2022). The historical use of plants such as *Cinchona* and *Artemisia annua* has been essential in the development of antimalarial drugs like quinine and artemisinin, respectively (Uchegbu et al., 2023). However, the increasing emergence of drug-resistant strains of *P. falciparum* remains a formidable challenge to malaria control (WHO, 2022). In Nigeria, resistance to earlier treatments such as

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chloroquine and sulphadoxine-pyrimethamine has rendered these options largely ineffective (Federal Ministry of Health, 2023). While artemisinin-based combination therapies (ACTs) are the current gold standard, their high cost and occasional unavailability limit their widespread use. Nigeria's diverse flora offers a promising avenue for discovering affordable, plant-based antimalarial therapies. This study seeks to evaluate the effectiveness of selected indigenous medicinal plants as part of combination therapy for malaria treatment. By combining drugs with different mechanisms of action, combination therapies reduce the risk of the parasite developing resistance to a single drug (Dondorp et al., 2023). Furthermore, the use of two or more drugs with synergistic effects can provide a broader therapeutic window, increasing treatment success rates in diverse populations, including children and pregnant women (Ashley et al., 2022). One of the challenges of combination therapies is that it can have side effects, which may lead to poor adherence to the full course of treatment, undermining its effectiveness (Smith et al., 2022).

Ageratum conyzoides (goat weed) is a widely distributed herbaceous plant belonging to the family Asteraceae, traditionally used in various regions for its medicinal properties. The indigenous Yorubas, Igbos and Hausas of Nigeria call it Imiesu, Ula ujula, and Ahenhen or Igedes respectively (Ufuopioko Onuoha et al., 2013). In Nigeria, it is known for its application in treating malaria, stroke, heart disorders, diabetes, and wounds. Research has demonstrated its antimicrobial, analgesic, antispasmodic, and anti-arthritic activities, supporting its traditional use in managing malaria and other ailments (Okunade, 2002; Asomugha et al., 2015).

Chromolaena odorata is a perennial shrub, also of the Asteraceae family, commonly used in Southeast Nigeria for its antimalarial and wound healing properties. The plant is commonly known as Siam weed, 'Elizabeth', 'Independence leaf' and 'Awolowo' among the Igbos of the South-Eastern Nigeria. Several studies have highlighted its broad pharmacological activities, including antibacterial, antifungal, antidiabetic, anti-inflammatory, and antioxidant effects. Its effectiveness against malaria and other diseases has been validated in several research studies (Asomugha et al., 2015; Adedapo et al., 2016).

Setaria megaphylla is a robust perennial grass traditionally employed in Nigeria for treating malaria, inflammation, and diabetes. Its common names are ribbon grass, and broad-leafed bristle grass. It is referred to by various local names in Nigeria, including "Eruwon" in Yoruba, "Ogba" in Igbo, and "Kurna" in Hausa. Indigenous communities use its leaf decoctions as a sedative for coughs and a remedy for urino-genital infections. The plant has shown antiplasmodial, hypoglycaemic, anti-inflammatory, analgesic, and cytotoxic activities in scientific studies, confirming its potential as an antimalarial agent (Burkill, 1985; Okokon et al., 2009).

Given the limitations of current antimalarial treatment options, there is a growing need to explore indigenous plants for their potential as affordable and effective antimalarial agents. This study evaluated the efficacy of *S. megaphylla*, *A. conyzoides*, and *C. odorata* individually and their combinations towards development of novel antimalarial therapies that are accessible and sustainable.

2. Materials and Methods

2.1. Plant Materials

Healthy fresh leaves of *S. megaphylla, A. conyzoides* Linn. and *C. odarata* Linn. were obtained from a farm at Ihiagwa community, Owerri West Local Government Area of Imo State, Nigeria. The plants were identified and authenticated by Mr. Francis Iwueze, a plant taxonomist of the Department of Forestry and Wild life, School of Agricultural Technology, FUTO with voucher numbers 722, 723 and 724 appropriately assigned and documented.

2.2. Preparation of Plant Samples

The fresh leaves were separately washed under running water, and dried under shade in the laboratory. The dried leaf samples were separately ground into powder using an electric grinder (Saisho 200W) and labelled. Each (500 g) was subjected to hot (100 ℃) aqueous extraction using deionized water (1000 ml). The aqueous extractions of the processed plant samples were carried out in single, binary (1:1 ratios) and ternary (1:1:1) combinations. Each aqueous extract was concentrated, labelled and stored at -4^{o}C until required for the antiplasmodial effect.

2.3. *In vitro* antioxidant study

In vitro antioxidant study involved determination of the phenolic profile, total antioxidant capacity and reducing power potential, as well as the free radical scavenging properties of the processed plant leaf samples.

2.4. Phenolic profile analysis

Quantitation of the plants' phenolic compounds was performed on a GC-FID system (Buck Scientific M910). The GC was equipped with an HP-5MS of 30 m length and 0.25 mm internal diameter capillary column, with 0.25 µm film thickness. The carrier gas was helium set to flow at 1.5 ml/min. The injector was operated in splitless mode at 280°C temperature. The chromatographic working conditions were optimized for the complete separation of the target compounds. The oven was programmed from 120°C (3.0 min) to 315°C with 5 °C/min and maintained for 5.0 minutes. The concentrations (µg/ml) of the phenolic compounds were resolved from the ratio of the mass and area of the internal standard to the area of the identified compounds.

2.5. Antioxidant potential

The total antioxidant capacities of the plants' aqueous leaf extracts were determined using phosphomolybdenum method (Prieto *et al.,* 1999), while their reducing power were assessed according to the method of Oyaizu (1986).

2.6. Free Radical Scavenging potential

The scavenging effect of the plants' extract on nitric oxide and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals were measured according to the methods described by Alisi & Onyeze (2008) and Velazquez *et al* (2003) respectively. The hydroxyl radical scavenging effect was analysed using free radical dependent 2-deoxyribose degradation method via the Fenton oxidant reaction mixture of Fe^{3+}/ascorbic acid and H_2O_2 as described by Halliwell *et al.* (1987).

2.7. LD_{50} Test

The study was carried out using ternary combination of the extracts in two phases, and involved 21 albino mice which were distributed into 7 groups of 3 animals each. In the phase 1, nine animals were divided into three groups and were orally administered different doses (10, 100 and 1000 mg/kg body weight) of test substance. The remaining 4 groups of the animals were used for the phase 2, and they were treated with 1000, 1600, 2900 and 5000 mg/kg body weight of the extract. At the end of each treatment, the animals were placed under observation for 24 hours to monitor for mortality and their behavior (Lorke, 1983). Then the LD_{50} value was calculated as:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D_0 = Highest dose that gave no mortality; and D_{100} = Lowest dose that produced mortality.

2.8. Antiplasmodial study

Fifty (50) Swiss albino mice and *Plasmodium berghei* NK 65 used for this study was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Nigeria, and transported to Federal University of Technology Owerri (FUTO) in liquid nitrogen; where the research was carried out. The mice were acclimatized for 2 weeks and the parasite, *Plasmodium berghei* NK 65 was maintained by sub-passaging into healthy mice via an intraperitoneal route as earlier described (Ene et al., 2008). The infection of the recipient mice was effected by needle passage of the parasite from the donor to healthy test animals via intraperitoneal route (David *et al*. (2004), Peter and Anatoli (1998), Ene et al*.,* 2008b).

The study was approved by the Ethics Committee of the Federal University of Technology Owerri, Nigeria.

2.9. *In vivo* Culture of the *P. berghei* using Albino Mice

P. berghei infected red blood cells (RBC) were collected via the tail and then diluted with phosphate buffer saline (PBS) pH 7.2 in such a way that each 0.2 ml had approximately 10 x 10^7 infected red blood cells (parasites) per kg of body weight. The diluted infected red blood cells were intraperitoneally injected into fresh healthy mice. The mice, both infected and uninfected, had free access to standard mice feedstuff (Vital Starter®) and water *ad libitum*, and were kept under standard laboratory condition with subsequent check. Parasitemia was confirmed in the animals after 24 hours of infection, by making thick blood smears from the tail vein of the infected mice, staining with Giemsa and viewing under microscope at x100 objective.

2.10. *In vivo* Treatment of *Plasmodium berghei* NK 65-Infected Mice with Plant Extracts

A four-day curative treatment study was conducted to evaluate the antimalarial efficacy of the plant extracts in Swiss albino mice infected with *Plasmodium berghei* NK 65. The study followed the protocols of David *et al*. (2004), Peter and Anatoli (1998), and Ene *et al*. (2008b). The infected mice were divided into eight groups, with five mice per group.

Forty-eight hours post-infection, the experimental groups received daily intraperitoneal doses of the plant extracts at 100 mg/kg body weight for four days. The dose was determined based on the extracts' LD_{50}. Control groups were treated with artesunate (1.6 mg/kg), chloroquine (10 mg/kg), or ACT (1.6 mg/kg), while a negative control group received no treatment.

At the end of the treatment period, blood smears were prepared from the mice, fixed with methanol, stained with Giemsa, and examined microscopically at x100 magnification under immersion oil. The percentage parasitemia was calculated according to the technique outlined by Iwalewa *et al.,* (1997) as:

$$Percentage\ parasitemia = \frac{\text{Number of parasites in treated}}{\text{Number of parasites in control}}\ x\ 100$$

This is always assumed to be:

$$Percentage\ parasitemia = \frac{\text{Number of parasites in treated}}{500}\ x\ 100$$

2.11. Statistical Analysis

The results generated were presented as mean ± standard deviation and analyzed using one-way analysis of variance and Duncan's postHoc test with the aid of Statistical Package for Social Sciences (SPSS, version 22). Statistical significance of values were considered at $p<0.05$.

3. Results

Results of the phenolic profile of the leaf extracts of *A. conyzoides, S. megaphylla, and C. odorata* show total phenolic contents to be 73.01, 118.06, and 116.58 µg/ml, respectively (Table 1). There were significantly higher contents of dihydrocytisine (13.00 and 13.24 µg/ml) and catechin (13.23 and 13.89 µg/ml) in *S. megaphylla, and C. odorata* respectively compared to *A. conyzoides.* A similar trend was observed for narigenin, epihedrine, steroid, and proanthocyanidin. Generally, tannin and spartein have comparable values in the three plant leaves analysed.

Figure 1A shows that the total antioxidant capacities (TAC) of the plants' leaf extracts increased with increase in extract concentration. *S. megaphylla* (1.49 ± 0.02 mg AAE/g) followed by *A. conyzoides* (1.24 ± 0.04 mg AAE/g) at the highest extract concentration of 400 µg/ml showed higher TAC values. Similarly, there were significant increase in reducing power potentials of the plant leaves with increase in extract concentration (Figure 1B). However, *S. megaphylla* and *C. odorata* at the highest extract concentration of 100 µg/ml showed significant ($p<0.05$) higher reducing power potentials at 0.47 ± 0.01 and 0.39 ± 0.03 mg AAE/g respectively.

Figures 2A – 2C show the hydroxyl, nitric oxide and DPPH radicals scavenging potentials of the plants leaf extracts. The results showed concentration dependent increases in radical scavenging potentials. At the highest extract concentration of 400 µg/ml, there were no observed significant ($p>0.05$) differences in the plants' percent inhibition of hydroxyl (68.78 ± 1.26, 76.10 ± 0.71 and 72.77 ± 3.89 %), nitric oxide (77.23 ± 2.67, 80.08 ± 4.92 and 69.10 ± 9.58 %), and DPPH (88.62 ± 2.34, 85.85 ± 2.85 and 90.05 ± 2.80 %) radicals for *A. conyzoides, S. megaphylla* and *C. odorata* respectively.

The acute toxicity experiment was conducted in two phases. In phase 1, the initial screening was at doses 10, 100 and 1000 mg/kg body weight, and no death occurred at doses 10 and 100mg/kg, while at dose 1000 mg/kg one death occurred. In phase 2, the screening was done at dose 1000, 1600, 2900 and 5000 mg/kg and the number of deaths were 1, 2, 3 and 3, respectively.

Table 3 presents the average percent parasitemia of the treated animal groups. The result shows that the parasitemia levels of the animal groups treated with *A. conyzoides* (AC), *C. odorata* (CO), and *S. megaphylla* (SM) singly varied from 2.12 ± 0.60 to 2.85 ± 1.59 %, 1.40 ± 0.49 to 1.90 ± 1.27 %, and 1.68 ± 0.50 to 1.10 ± 0.42 % respectively for the 0 – 13 days treatment. On the other hand, the binary combinations elicited parasitemia variations of 1.60 ± 0.35 to 0.50 ± 0.14

%, 2.40 ± 0.98 to 0.40 ± 0.00 %, and 2.00 ± 0.71 to 0.40 ± 0.00 % for AC+CO, AC+SM and CO+SM respectively, while the ternary combination gave values of 1.84 ± 0.26 to 2.00 ± 0.00% representing the 0 to 13 days of treatment. The artimisinin combination therapy standard (ACT STD) drug gave a complete parasite eradication by day 5, while the chloroquine control (CQ CTRL) had percent parasitemia values comparable with those of the AC+SM, but less effective compared to CO+SM.

Table 1 Phenolic profile of leaf extracts of *Ageratum conyzoides* Linn, *Setaria megaphylla* and *Chromolaena odarata* Linn

Component (µg/ml)	***Ageratum conyzoides***	***Setaria megaphylla***	***Chromolaena odarata***
Sapogenin	1.47	1.15	1.80
Narigenin	2.45	8.22	8.17
Anthocyanin	4.01	7.64	3.91
Epihedrine	1.86	6.82	7.22
Dihydrocytisine	2.91	13.00	13.24
Kaempferol	4.11	4.06	4.33
Cyanogenic glycoside	1.27	1.34	1.44
Aphyllidine	1.30	4.74	5.09
Steroid	3.10	8.16	8.84
Tannin	10.97	12.17	12.86
Flavonones	3.52	6.59	4.53
Catechin	3.49	13.23	13.89
Flavone	3.58	3.32	3.61
Proanthocyanidin	2.30	7.84	7.90
Ribalinidine	3.49	6.78	5.64
Spartein	8.80	7.74	8.68
Cardiac glycoside	8.40	ND	ND
Phytate	5.98	ND	ND
Ammodendrine	ND	1.78	1.78
Oxalate	ND	3.48	3.65
TOTAL	73.01	118.06	116.58

*ND not detected

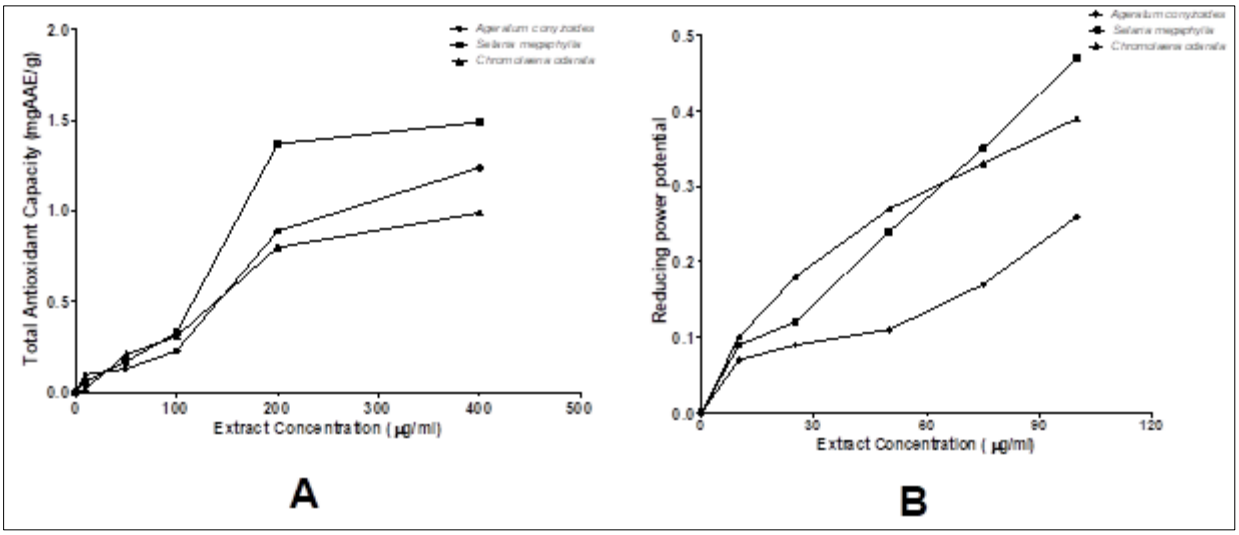

Figure 1 Total antioxidant capacity (A) and reducing power potential (B) of the leaf extracts of *Ageratum conyzoides* Linn, *Setaria megaphylla* and *Chromolaena odarata* Linn

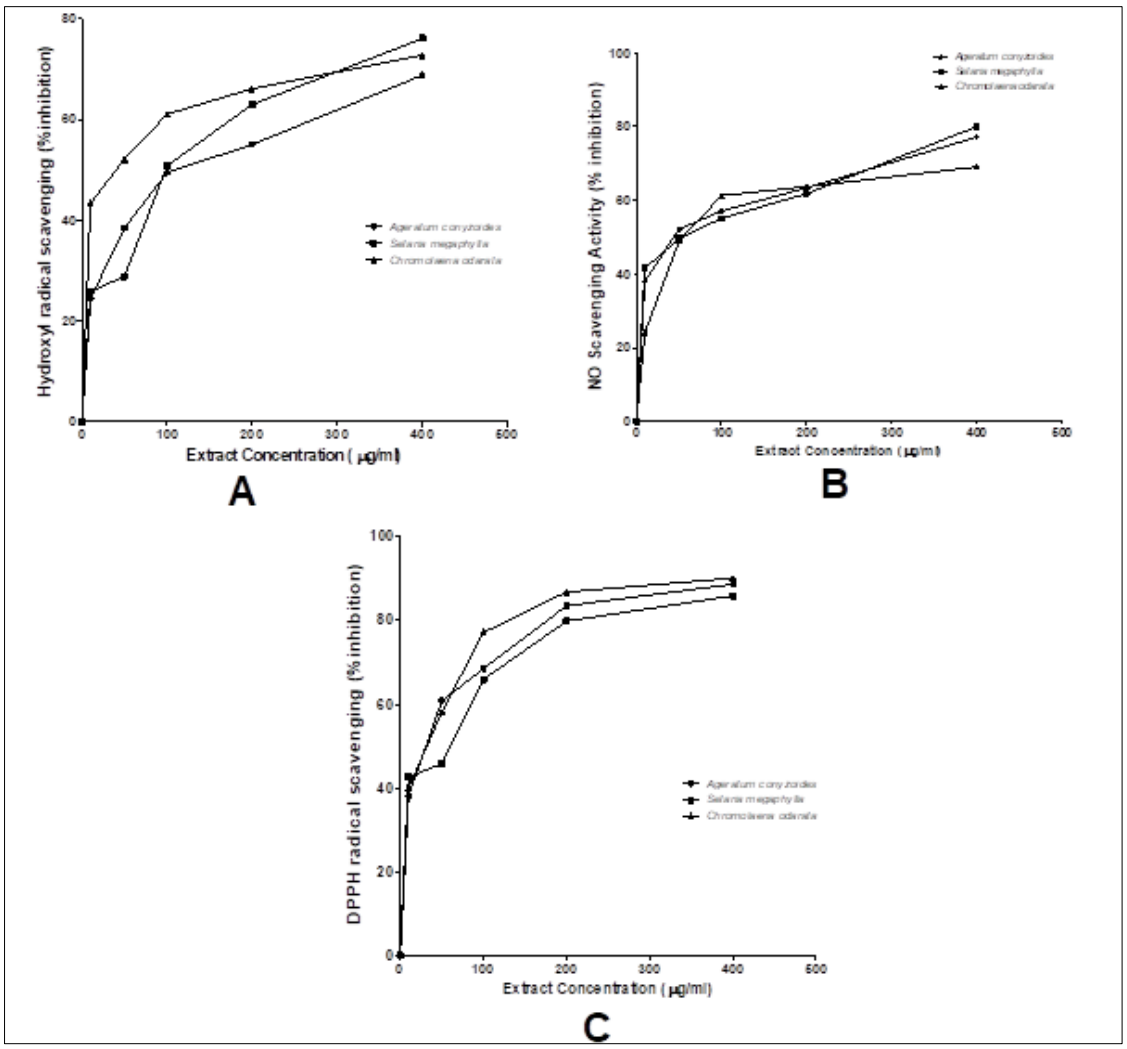

Figure 2 Hydroxyl (A), nitric oxide (B) and DPPH (C) radicals scavenging potentials (% inhibition) of the leaf extracts of *Ageratum conyzoides* Linn, *Setaria megaphylla* and *Chromolaena odarata* Linn

Table 2 Median lethal dose (LD50) of ternary combination of leaf extracts of *Ageratum conyzoides* Linn, *Setaria megaphylla* and *Chromolaena odarata* Linn

Groups	Dose of tea extract (mg/kg)	Number of death recorded
Phase 1		
GP 1	10	0/3
GP 2	100	0/3
GP 3	1000	1/3
Phase 2		
GP 1	1000	1/3
GP 2	1600	2/3
GP 3	2900	3/3
GP 4	5000	3/3

$LD_{50} = \sqrt{100 \times 1000} = 316.23$ mg/Kg body weight

Table 3 Average parasitaemia levels (%) of infected animal groups treated with leaf extracts of *Ageratum conyzoides* Linn, *Setaria megaphylla* and *Chromolaena odarata* Linn

Animal Groups	Level of parasitaemia (Days)													
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
AC	2.12 ± 0.66[a]	2.72 ± 1.00 a	1.55 ± 0.90 ab	1.15 ± 0.57 ab	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac	2.85 ± 1.59 ac
CO	1.40 ± 0.49 a	2.40 ± 0.42 a	1.56 ± 0.33 ab	1.50 ± 0.27 ab	1.50 ± 0.27 ab	3.20 ± 1.13 a	1.70 ± 0.99[a]	1.90 ± 1.27 a	1.90 ± 1.27 a	1.90 ± 1.27 a	1.90 ± 1.27 a	1.90 ± 1.27 a	1.90 ± 1.27 a	1.90 ± 1.27 a
SM	1.68 ± 0.50 a	2.60 ± 0.63 a	2.10 ± 0.81 ab	2.33 ± 0.23 ab	1.50 ± 0.71 ab	2.10 ± 0.14 ac	1.60 ± 0.57 a	1.50 ± 0.71 a	1.10 ± 0.42 a	1.10 ± 0.42 a	1.10 ± 0.42 a	1.10 ± 0.42 a	1.10 ± 0.42 a	1.10 ± 0.42 a
AC+CO	1.60 ± 0.35 a	1.80 ± 0.35 a	1.52 ± 0.77 ab	1.30 ± 0.50 ab	0.60 ± 0.23 b	0.60 ± 0.16 b	0.80 ± 0.57 a	0.70 ± 0.14 a	0.50 ± 0.42 a	0.50 ± 0.14 a	0.50 ± 0.14 ab	0.50 ± 0.14 a	0.50 ± 0.14 a	0.50 ± 0.14 a
AC+SM	2.40 ± 0.98 a	1.72 ± 0.18 a	1.68 ± 0.54 ab	1.88 ± 0.81 ab	2.12 ± 0.46 abc	1.90 ± 0.38 bc	1.70 ± 0.35 a	1.60 ± 0.43 a	1.25 ± 0.25 a	0.65 ± 0.19 a	0.55 ± 0.10 ab	0.55 ± 0.10 a	0.53 ± 0.12 a	0.40 ± 0.00 a
CO+SM	2.00 ± 0.71 a	2.36 ± 0.62 a	1.16 ± 0.36 b	2.65 ± 1.05 ac	1.85 ± 0.77 ab	1.05 ± 0.25 bc	1.13 ± 0.12 a	0.87 ± 0.31 a	0.60 ± 0.20 a	0.40 ± 0.00 a	0.40 ± 0.00 a	0.40 ± 0.00 a	0.40 ± 0.00 a	0.40 ± 0.00 a
AC+CO+SM	1.84 ±	2.16 ±	1.10 ±	1.00 ±	3.33 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±	2.00 ±

	0.26 a	1.22 a	0.38 b	0.20 b	0.92 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
ACT STD	2.52 ± 1.08 a	2.64 ± 0.62 a	1.28 ± 0.41 bc	0.92 ± 0.41 b	0.64 ± 0.30 b	0.00 ± 0.00 d	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
CQ CTRL	2.00 ± 0.32 a	2.40 ± 0.51 a	2.76 ± 0.36 ac	2.12 ± 0.44 ab	0.80 ± 0.57 b	1.20 ± 1.12 bc	0.75 ± 0.38 a	1.00 ± 0.54 a	0.73 ± 0.42 a	0.60 ± 0.20 a	0.60 ± 0.00 b	0.60 ± 0.00 a	0.40 ± 0.00 a	0.40 ± 0.00 a
UNT CTRL	2.04 ± 0.67 a	2.16 ± 0.48 a	2.84 ± 1.00 a	4.60 ± 1.18 c	3.13 ± 0.50 C	1.13 ± 0.42 bc	1.93 ± 1.63 a	1.00 ± 0.53 a	1.00 ± 0.53 a	1.70 ± 0.14 c	1.70 ± 0.14 c	1.70 ± 0.14 c	1.70 ± 0.14 c	1.70 ± 0.14 c

Values are mean ± standard deviation. Values with different superscript letters per column are statistically significant ($p<0.05$). n = 5. Where AC = *Ageratum conyzoides,* SM = *Setaria megaphylla*, CO = *Chromolaena odarata Linn,* ACT STD = Artesunate standard control, CQ CTRL= Chloroquine control, and UNT CTRL= untreated control.

4. Discussion

The assessment of the phenolic profile of leaf extracts from *Ageratum conyzoides Linn*, *Setaria megaphylla*, and *Chromolaena odorata Linn* is vital in understanding their antimalarial potential. Phenolic compounds are renowned for their antioxidant, anti-inflammatory, and antimicrobial properties, which are key contributors to their effectiveness in treating malaria (Aworet-Samseny et al., 2020). Among these plants, *S. megaphylla* exhibits the highest total phenolic content, with significant amounts of catechins, dihydrocytisine, and tannins. Catechins, known for their potent antioxidant properties, have been shown to inhibit hemozoin formation in malaria parasites, making them effective antimalarials (Akinmoladun et al., 2021). Additionally, dihydrocytisine and naringenin contribute to strong antimalarial bioactivity; dihydrocytisine displays substantial antiparasitic effects, while naringenin is recognized for its anti-inflammatory properties (Ejeh et al., 2022). *C. odorata* has a phenolic content similar to *S. megaphylla* and shares many of these key compounds. High levels of catechins, dihydrocytisine, and tannins in these plants are indicative of their strong antimalarial potential, with naringenin and steroidal compounds further enhancing their medicinal value through anti-inflammatory and antiparasitic effects (Aliyu et al., 2023).

In addition, the presence of tannins, sparteine, and cardiac glycosides in the leaf extracts of these plants adds to their antimalarial efficacy. Tannins are known for their potent antioxidant properties, helping to mitigate oxidative stress caused by malaria parasites (Ndhlala et al., 2020). Sparteine, a well-known antiparasitic agent, boosts the extracts' antimalarial potential (Ogidi et al., 2021). Though cardiac glycosides are primarily recognized for their cardiovascular effects, they have been found to disrupt parasite cellular processes, enhancing their antimalarial action (Onyeagba et al., 2019). The therapeutic potential of these phenolic compounds is well established, with studies documenting the antimalarial properties of tannins, catechins, and flavonoids due to their ability to inhibit *Plasmodium falciparum* growth and reduce oxidative stress (Suleiman et al., 2021; Ajayi et al., 2023).

The total antioxidant capacity (TAC) is a critical metric for assessing a plant's potential in antimalarial applications, as antioxidants help mitigate oxidative stress caused by malaria, thereby enhancing the efficacy of treatments (Akinmoladun et al., 2021). *Ageratum conyzoides* demonstrated a dose-dependent increase in antioxidant capacity, reaching a peak value of 1.24 mg AAE/g at 400 µg/ml, suggesting its antioxidant properties improve with concentration. Previous studies indicate that phenolic compounds, such as tannins and flavonoids present in *A. conyzoides*, contribute significantly to its antioxidant capacity, neutralizing free radicals and reducing oxidative stress (Aliyu et al., 2023). These compounds make the plant a valuable candidate for antimalarial treatments (Suleiman et al., 2021).

Setaria megaphylla exhibited the highest antioxidant capacity of the three plants, with a maximum of 1.49 mg AAE/g at 400 µg/ml, highlighting its strong potential in mitigating oxidative stress associated with malaria. The high levels of catechins and dihydrocytisine in *S. megaphylla* are likely responsible for its robust antioxidant activity, as these compounds are known for their potent free radical scavenging abilities (Ejeh et al., 2022). *Chromolaena odorata* showed moderate antioxidant capacity in comparison, with a TAC of 0.99 mg AAE/g at 400 µg/ml. While catechins and dihydrocytisine were present, their levels were not as high as in *S. megaphylla*, resulting in a lower antioxidant capacity.

Nevertheless, its moderate TAC still suggests a potential role in antimalarial therapy, albeit less potent than *S. megaphylla* (Ogidi et al., 2021).

The reducing power potential of plant extracts is also a key indicator of their antioxidant capacity, correlating directly with their ability to neutralize free radicals and enhance antimalarial efficacy (Ndhlala et al., 2020). The reducing power observed in this study is consistent with previous findings that highlight the importance of phenolic compounds, such as catechins and dihydrocytisine, in determining the antioxidant potential of plant extracts (Akinmoladun et al., 2021). Similarly, the moderate reducing power of *C. odorata* aligns with other research emphasizing the role of anthocyanins and tannins in boosting antioxidant properties (Suleiman et al., 2021).

Hydroxyl radicals are highly reactive species that contribute significantly to oxidative stress, which plays a major role in the pathogenesis of malaria. The scavenging of hydroxyl radicals by plant extracts is a key indicator of their antioxidant capacity and potential therapeutic effects against malaria (Akinmoladun et al., 2021). As illustrated in Figure 2A, *Ageratum conyzoides* exhibited a marked increase in hydroxyl radical scavenging activity with concentration, achieving a maximum inhibition of 68.78% at 400 µg/ml. This high level of activity is likely due to the presence of phenolic compounds, such as tannins and flavonoids, known for their potent antioxidant properties (Suleiman et al., 2021). *Setaria megaphylla* demonstrated the highest hydroxyl radical scavenging potential, with a maximum inhibition of 76.10% at 400 µg/ml, attributable to its high levels of catechins and dihydrocytisine, which are recognized for their robust antioxidative effects (Ejeh et al., 2022). These findings are consistent with previous studies that have confirmed the strong antioxidant capacity of *S. megaphylla* (Aliyu et al., 2023). Similarly, *Chromolaena odorata* displayed a significant hydroxyl radical scavenging activity, achieving a maximum inhibition of 72.77% at 400 µg/ml, which is likely linked to its substantial anthocyanin and tannin content (Ngameni et al., 2020).

Nitric oxide (NO) radicals serve dual roles in biological systems, acting as signaling molecules at low concentrations but contributing to oxidative stress and inflammation at higher levels. The ability of plant extracts to scavenge NO radicals is a critical indicator of their antioxidant and potential therapeutic properties (Suleiman et al., 2021). The results of the NO radical scavenging activity indicate that *S. megaphylla* possessed the highest antioxidant capacity, followed closely by *A. conyzoides* and then *C. odorata*. These findings suggest that the plants have considerable therapeutic potential in mitigating oxidative stress and inflammation, which are critical in malaria pathogenesis. The NO scavenging potentials observed are consistent with previous research, highlighting the role of phenolic compounds in enhancing the antioxidant properties of plant extracts (Ejeh et al., 2022). The high scavenging activity of *S. megaphylla* correlates with its rich phenolic content, particularly catechins, known for their antioxidative properties (Aliyu et al., 2023).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely used and reliable method for assessing the antioxidant capacity of plant extracts. The ability of plant extracts to donate hydrogen atoms to neutralize DPPH radicals is indicative of their overall antioxidant potential (Ndhlala et al., 2020). The DPPH scavenging results in this study demonstrate that all three plant extracts have significant antioxidant properties, with *C. odorata* showing the highest scavenging potential, followed by *A. conyzoides* and *S. megaphylla*, although the differences in their activity at the highest extract concentration were not statistically significant. These findings suggest that the plants could play an important role in managing oxidative stress-related conditions, such as malaria, due to their potent antioxidant effects (Suleiman et al., 2021).

Acute toxicity study of the ternary combination of the plants' extracts showed an LD_{50} of 316.23 mg/kg suggesting that the extracts in combination, and by extension individually, has moderate level of toxicity. This value falls within a range that indicates the extract is not highly toxic. This further buttresses the fact that the plants serve as folder for animals, and have been locally administered to humans for treatment of several ailments (Asomugha et al., 2015; Adedapo et al., 2016).

The data presented in Table 3 showed the antiplasmodial efficacy of the various plant extracts and their combinations against *P. berghei* NK 65 in infected albino mice. The treatment was administered intraperitoneally for four days, starting 48 hours post-infection, with a standard artesunate, chloroquine, and ACT as controls. In group treated with *A. conyzoides* (AC), the parasitemia levels showed a steady increase from day 0 (2.12 ± 0.60 %) to day 13 (2.85 ± 1.59 %) instead of expected reduction. The extract did not demonstrate a significant reduction in parasitemia compared to the untreated control (UNT CTRL). For *C. odarata* (CO) treated group, similar to AC, CO exhibited fluctuating parasitemia levels without significant reduction. There was an initial increase in parasitemia (2.40 ± 0.42 % on day 1), which later reduced to 1.90 ± 1.27 % on day 7 when death occurred. *S. megaphylla* (SM) exhibited a slightly better parasitemia control, reducing to 1.10 ± 0.42 % by day 7 and remained same up to day 13. For the binary combination treatments, AC+CO combination showed a marked reduction in parasitemia to 0.50 ± 0.14 % by day 9 suggesting a synergistic effect, but the death of animal followed on day 10, thus questioning its efficacy. Similarly, CO+SM showed significant

parasitemia reduction achieving 0.40 ± 0.00 % with death on day 10. AC+SM demonstrated the best significant reduction in parasitemia to 0.40 ± 0.00 % from day 9 through day 13, without any death occurring, indicating improved efficacy over single and other combined extracts. For the ternary combination (AC+CO+SM), despite the initial decrease in parasitemia, levels increased again, showing inconsistency in the therapeutic effect, with death occurring on day 6. Thus, AC+SM binary combination offers better parasitemia control than the single and other combination extracts. Nogueira *et al*. (2019) emphasized the potential of combination therapies using plant extracts to enhance antimalarial efficacy and prevent resistance. Also, Mekonnen *et al*. (2021) investigated various plant extracts and found that combinations provided superior therapeutic outcomes compared to monotherapies. However, it is important to note that complete eradication of parasitemia was not achieved with any of the extracts when compared with the use of artesunate, hence further study and research is encouraged

5. Conclusion

The leaf extracts of *S. megaphylla*, *A. conyzoides*, and *C. odorata* exhibited significant antioxidant and moderate antimalarial properties. Among the tested extracts, the combination of *A. conyzoides* and *S. megaphylla* showed the most promising results in reducing parasitemia in *P. berghei*-infected mice, suggesting a potential synergistic effect that could be beneficial in antimalarial therapy. However, the inability to achieve complete parasitemia clearance underscores the need for further research to optimize extract combinations and dosages. Future studies should explore the potential of these plant extracts in combination with standard antimalarial drugs to enhance therapeutic outcomes and mitigate resistance. The results contribute to the growing body of evidence supporting the use of plant-based therapies in malaria treatment, particularly in regions where drug resistance is a significant concern.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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