



Chemical Substances and *in-Vivo* Antiplasmodial Activity of *Ageratum Conyzoides* in *Plasmodium Berghei* Infected Mice

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ABSTRACT: Malaria afflicts millions of people globally, particularly in tropical Africa; it is transmitted to humans through a bite of an Anopheles mosquito. Phytochemical, acute toxicity and in-vivo antiplasmodial activity of the leaves of *Ageratum conyzoides* were examined to study its effects on Mice that have been infected with the malaria parasite. Phytochemical screening of the methanol extract revealed the presence of secondary metabolites such as terpenoids, flavonoids, alkaloids, steroids and chromene. The LD50 was established at > 1000 mg/kg body weight of mice. The methanol extract of *A. conyzoides* displayed intrinsic prophylactic and curative anti-malaria activity. At 200 mg/kg and 100 mg/kg body weight of mice, the extract revealed the highest percentage inhibition (83 and 61) for the prophylactic and curative study respectively. The acute toxicity study showed that *A. conyzoides* extract is relatively safe within the study administered doses. The methanol extract of the prophylactic study against *Plasmodium berghei* revealed an increase in the level of significance at administered portions of 100, 200 and 400 mg/kg in comparison with 0.2 ml distilled water and 10 mg/kg chloroquine. The methanol extract of the therapeutic study against *Plasmodium berghei* revealed a slight increase in the level of significance at administered doses of 100 and 200 mg/kg, however, no significant effect was observed for 400 mg/kg compared to the negative control and reference drug. The outcome implies that methanol leave extract of *A. conyzoides* possesses meaningful antiplasmodial activities and could be a promising source of novel antimalarial.

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Malaria is produced by a parasite called plasmodium, which is transferred by means of bites of contaminated mosquito (Okethwangu *et al.*, 2019). The parasite reproduces in the liver of a human body and there-after taint the red blood cells (Malawi, 2002). Between 10 – 15 days after a mosquito bite, malaria indication such as fever, headache, and vomiting initiate notice in an infected person (Malawi, 2002). Malaria is a mosquito-borne protozoan illness that is a main public health distress throughout the world. The group of plasmodia identified to infect humans are; *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* (Ortiz-Ruiz *et al.*, 2018). The valuable anti-malaria action of the two plant-based drugs against *Plasmodium falciparum*; quinine and artemisinin have produced much curiosity to discover other plants properties for their possible anti-malaria efficacy (Mishra *et al.*, 2009). The need to search and build up more effective anti-malaria drugs that are low-cost and readily obtainable to people in developing countries like Nigeria and some other countries in the sub-Saharan regions has necessitated this study. *Ageratum*

is derived from the Greek word ‘Ageras’, meaning non-aging, referring to the permanence of the whole plant. *Conyzoides*, on the other hand is derived from ‘Konyz’ the Greek term of ‘inutahelenium’ which the plant look like (Novaes *et al.*, 2013; Okunade, 2002). The weed of *A. conyzoides* has been known for centuries, for its therapeutic properties and has been employed for treatment of different health conditions, such as wounds and burns, for antimicrobial and anti-malaria activities, for many bacteria and fungi infections, antitetanus, arthrosis, headaches and dyspnea, pneumonia, analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases (Santos *et al.*, 2016). A wide range of chemical compounds including flavonoids, chromenes, alkaloids, cumarins, benzofurans, terpenoids and sterols, have been isolated from this species. Extracts and metabolites from this plant have been established to have some insecticidal and pharmacological actions (Santos *et al.*, 2016; Ukwe Chinwe *et al.*, 2010).

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In eastern Nigeria, aqueous extracts of leaves of *Ageratum conyzoides* is utilized by herbalists and individuals to treat malaria. In light of the above, this study was set to establish the pharmacological basis for the apparent anti-malaria activity of leaves of *A. conyzoides*. In Nigeria, various tribes have diverse names for it. For instance, Igede of the middle belt, Yorubas of the south west, and Igbos of the south east of the country call it "Ufuopioko", "Imiesu", and "Nriewu" respectively (Agbafor et al., 2015; Ukwé Chinwe et al., 2010). The need to search and build up a more effective anti-malaria drug that is low-cost and readily obtainable to people in developing countries like Nigeria and some other countries in the sub-Saharan regions has necessitated this study. The aim of the study was to evaluate the phytochemical screening and antiplasmodial activity of *A. conyzoides* in mice.

MATERIALS AND METHOD

General experimental: Specific-pathogen-free, female and male mice (*Mus musculus*), aged 6-8 weeks old and weighed 30-35 g were used. Chloroquine-sensitive *Plasmodium berghei* strain ANKA (PbANKA) was used. The mice were apportioned into five (5) groups, five (5) animals per group for prophylactic. Group 1: 10 mg/kg Chloroquine, Group 2: 0.2 ml Distilled water Group 4: 100 mg/kg aqueous plant extract, Group 5: 200 mg/kg aqueous plant extract, Group 6: 400 mg/kg aqueous plant extract five animals per group for curative.

Collection and preparation of *Ageratum conyzoides* leaves: The plant *A. conyzoides* was collected fresh from Adolo community, Benin City, Nigeria. Identification of the leaves was carried out at the Department of Plant Biology and Biotechnology, University of Benin and authenticated by Dr Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. A voucher specimen was prepared and herbarium specimen number UBHA344 was deposited. The leaves were rinsed with water after which they were air-dried and ground into a powder with the aid of a mechanical machine. It was stored in an airtight container until ready for use.

Extraction of Crude Powdered Sample: The fresh leaves of *A. conyzoides* were rinsed, shade dried and pulverized to coarse powder. The powdered plant material (900g) was extracted with 98% methanol using Soxhlet extractor to obtain crude methanol extract. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and the percentage yield calculated based on the initial weight of the crude powdered sample. The extract was stored in an air-

tight container and kept in the refrigerator at 4°C until further experiment.

$$\% \text{ Yield of AC} = \frac{\text{wt obtained}}{\text{initial wt of sample}} \times 100$$

$$\% \text{ yield of AC} = \frac{20.8400\text{g}}{900\text{g}} \times 100 = 2.316 \%$$

Where AC = *A. Conyzoides*

Phytochemical Screening: Phytochemical screening was performed on the methanol extract using simple chemical tests to detect the presence of secondary metabolite based on standard methods.

Acute toxicity study: Animals: Specific-pathogen-free, female and male mice (*Mus musculus*), aged 6-8 weeks old and weighted 30-35 g were purchased from the Department of Biochemistry Animal House, University of Benin, Nigeria. They were kept at 22-25°C with 12 h light/dark cycle and given standard mouse pellet and water ad libitum. They were acclimatized for 14 days. Procedures of the animal experiments were ratified by the Ethical Committee on Animal Experimentation, Faculty of Pharmacy, University of Benin.

Experimental: The acute toxicity test of aqueous crude extract of *A. Conyzoides* was carried out as previously described by Lorke (Lorke, 1983) with modifications. Groups of naïve ICR mice (5 mice of each) were given orally by gavage with 100, 500 and 1000 mg/kg. The mice were observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of the entire body, weight loss, weakness, respiratory distress and death in the first 4 h and subsequently daily for 7 days.

Anti-malaria drug: Standard anti-malaria drug, chloroquine diphosphate salt was used to study *in vivo* drug susceptibility of PbANKA. The drug was freshly prepared in distilled water and administered orally by gavage (Franke-Fayard et al., 2008) (Ifijen et al., 2019). Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of each mouse. The dose of 10 mg/kg was based on the ED90 of this drug on PbANKA infected mice.

Estimation of Percentage Parasitaemia: Percentage parasitemia was estimated at the end of the observational period using the formula

$$\% \text{ parasitemia} = \frac{\text{P. RBC}}{\text{P. RBC} \times \text{NP. RBC}} \times 100$$

Where P = parasitized; NP = Non-parasitized

Estimation of Mean Survival Time (MST): The number of days each animal survived was recorded for the animals in each group and mean survival time calculated using the formula

$$MST = \frac{\sum SA / Group}{TA}$$

Where $\sum SA$ = sum of days of survival of animal and TA = total number of animals in the group

RESULTS AND DISCUSSION

The qualitative and quantitative phytochemical screening of the aqueous extract of *A. conyzoides* revealed the presence of alkaloids, saponin, tannin, phenol, flavonoid, terpenoid, eugenol, steroid and glycoside (Table 1).

Table 1 Phytochemical Composition of the Leaves of *A. conyzoides*

Phytochemical	Inference
Glycoside	++
Steroids	++
Terpenoids	++
Alkaloids	++
Tanins	++
Flavonoids	++
Phenolics	++
Saponins	+
Eugenol	+

+: indicates presence of component; -: indicates absence of component; ++: indicates largely present of component

The plant flavonoids which are phenolic compounds are known to serve as flavouring ingredients of plant leaves. They have been found to possess an antioxidant potential in animals. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported anti-mutagenic and anti-carcinogenic effects (Middleton, 1998). Alkaloids have been implicated in the anti-malaria activity of many plants (Hamann, 2001; Nafiu et al., 2013). Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoans such as *P. falciparum* (Gebrehiwot et al., 2019). The presence of secondary metabolites, alkaloids and flavonoids in *A. conyzoides* has been implicated in the antiplasmodial effects of some herbal medicines (Phillipson and Wright, 1991). The behavioural pattern of acute toxicity study of the animals showed no abnormal behaviour within 24 hours of *A. conyzoides* extract exposure, and no mortality was observed all through the study, this showed that *A. conyzoides* extracts have no toxic effect

across the administered doses 100, 500 and 1000 mg/kg (Table 3).

Table 3: Effect of *A. conyzoides* extracts on acute toxicity study

Groups	Doses (mg/kg)	Number of Mice	Mortality (%)
<i>Ageratum conyzoides</i>	100	5	0
<i>Ageratum conyzoides</i>	500	5	0
<i>Ageratum conyzoides</i>	1000	5	0

A. conyzoides extract prophylactic study against *P. berghei* showed an increase in the level of significance for the graded administered doses (100, 200 and 400 mg/kg) of *A. conyzoides* in comparison with 0.2 ml Distilled water and 10 mg/kg Chloroquine of the parasitemia count. The result presented in Table 4 shows that the extract exhibited good anti-malaria properties. 100, 200 and 400 mg/kg were observed to have exhibited more sensitivity, specifically at 100 and 400 mg/kg. They were observed to be even more sensitive than the control groups with respect to the reduction of *Plasmodium berghei* as recorded from the observed percentage inhibition (10 mg/kg Chloroquine, 100 mg/kg, 200 mg/kg and 400 mg/kg of the extracts) at 76 %, 64 %, 83 % and 81 % (Table 4, Figure 1). A study of the survival days showed that the longest survival days was recorded by the 200 mg/kg dose. It was observed to be more than 10 mg/kg Chloroquine and negative control (7.67±0.89, 20.33±0.33, 20.0±1.15, 22.3±1.76, 18.67±0.88 and 7.67±0.89) sensitivity against *Plasmodium berghei* (Table 4). This was slightly different from the observation of Ukwe et al (Ukwe Chinwe V et al., 2010) as the Chloroquine percentage inhibition was higher than that of the Methanol extract (5mg/kg Chloroquine, 100mg/kg, 200mg/kg and 400mg/kg of the extract) at 82.60%, 43.91%, 56.52%, and 71.74% respectively. Curative study of *A. conyzoides* extract against *Plasmodium berghei* induced malaria showed a slight increase in the level of significance in 100 and 200 mg/kg of *Ageratum conyzoides*.

However, no significant effect was observed for 400 mg/kg compared to the negative control and reference drug with significant increase in the parasitemia count. The extract exhibited anti-malaria properties and standard drug drastically reduce the level *Plasmodium berghei* as recorded from the percentage inhibition in the graded doses of the treated plant (52 %, 61%, 47% and 37%) revealed in Table 5 and Figure 2. The surviving days showed average survival for the treated groups in comparison to the 10 mg/kg Chloroquine and negative control (7.67±0.88, 17.33±1.45, 12.33±0.88, 13.0±0.57 and 11.33±0.88).

Table 4: Effect of *A. conyzoides* extracts on prophylactic treatment of *P. berghei* induced malaria

Drugs	Doses (mg/kg)	Mean \pm SEM of Parasitemia	% inhibition	Mean \pm SEM of survival days
Distilled water	0.2 ml	1.00 \pm 0.00	0	7.67 \pm 0.89
Chloroquine	10	0.24 \pm 0.04 ^a	76	20.33 \pm 0.33
<i>A. conyzoides</i>	100	0.22 \pm 0.19 ^a	67	20.0 \pm 1.15
<i>A. conyzoides</i>	200	0.17 \pm 0.08 ^b	83	22.3 \pm 1.76
<i>A. conyzoides</i>	400	0.19 \pm 0.11 ^a	81	18.67 \pm 0.88

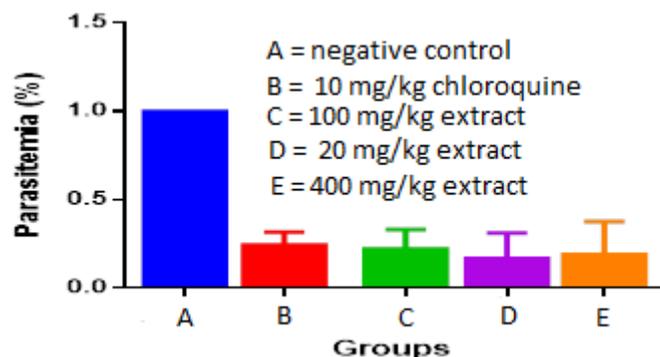
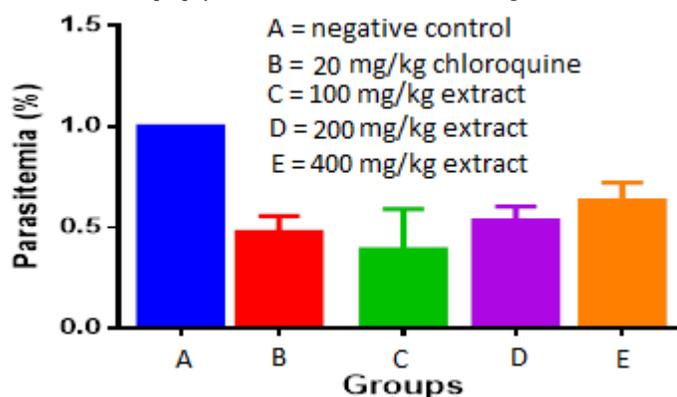
Table 5: Effect of *A. conyzoides* extracts on curative treatment of *Plasmodium berghei* induced malaria

Drugs	Doses (mg/kg)	Mean \pm SEM of Parasitemia	% inhibition	Mean \pm SEM of survival days
Distilled water	0.2 ml	1.00 \pm 0.00	0	7.67 \pm 0.88
Chloroquine	20	0.48 \pm 0.08 ^a	52	17.33 \pm 1.45
<i>A. conyzoides</i>	100	0.39 \pm 0.20 ^b	61	12.33 \pm 0.88
<i>A. conyzoides</i>	200	0.53 \pm 0.07 ^a	47	13.0 \pm 0.57
<i>A. conyzoides</i>	400	0.36 \pm 0.09	37	11.33 \pm 0.88

a=p<0.05, b=p<0.1 n=5

Table 2 Calculated Flavonoid and Phenolic values of methanol Extract

Flavonoid values (mg/g)	Phenolic value (mg/g)
48	251

**Fig. 1:** Effect of *A. conyzoides* extracts on prophylactic treatment of *Plasmodium berghei* induced malaria across the various groups**Fig. 2:** Effect of *A. conyzoides* extracts on curative treatment of *P. berghei* induced malaria across the various groups

Conclusion: The present study revealed that the aqueous leaf extract of *A. conyzoides* have antiplasmodial activity against experimentally induced plasmodiasis in laboratory animals. The extract appears to be potentially useful in the treatment of malaria. However more phytochemical studies are

required in order to isolate the active secondary metabolites in it.

REFERENCE

Agbafor K N, Engwa A G, Ude C M, Obiudu I K, and Festus B O (2015). The Effect of Aqueous Leaf Extract of *Ageratum Conyzoides* on Blood

- Glucose, Creatinine and Calcium Ion Levels in Albino Rats. *J. Pharma. Chem. Biol. Sci.* 3, 408-415.
- Agbafor, K. N., and Nwachukwu, N. (2011). Phytochemical Analysis and Antioxidant Property of Leaf Extracts of *Vitex doniana* and *Mucuna pruriens*. *Biochem. Res. Inter.* 4.
- Franke-Fayard, B., Djokovic, D., Dooren, M. W., Ramesar, J., Waters, A. P., Falade, M. O., Kranendonk, M., Martinelli, A., Cravo, P., and Janse, C. J. (2008). Simple and sensitive antimalarial drug screening in vitro and in vivo using transgenic luciferase expressing *Plasmodium berghei* parasites. *Int J Parasitol* 38, 1651-62.
- Gebrehiwot, S., Shumbahri, M., Eyado, A., and Yohannes, T. (2019). Phytochemical Screening and In Vivo Antimalarial Activity of Two Traditionally Used Medicinal Plants of Afar Region, Ethiopia, against *Plasmodium berghei* in Swiss Albino Mice. *J. Parasitol. Res.* 2019, 8.
- Hamann, M. T. (2001). Bioactive Compounds from Natural Sources: Isolation, Characterisation and Biological Properties Edited by Corrado Tringali (Universita di Catania, Italy). Taylor & Francis Publishing, London and New York. ISBN 0-7484-0890-8. *J. Nat. Prod.* 64, 1382-1382.
- Ifijen, I. H., Mamza, A. U., Fasina, K. A., Omoruyi, J. I., and Ikhuoria, E. U. (2019). Phytochemical Analysis of *Guiera senegalensis* J.F. Gmel Extract and its Anti-Plasmodial Properties on Wister Albino Mice via Oral Route. *Inter. J. Pharm. Phytochem. Ethnomed.* 13, 35-44.
- Kisangau, D. P., Lyaruu, H. V. M., Hosea, K. M., and Joseph, C. C. (2007). Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: a case in the Bukoba rural district. *J. Ethnobiol. Ethnomed.* 3, 29-29.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch Toxicol* 54, 275-87.
- Malawi, R. P. o. M. R. i. (2002). Malawi Medical Journal : *J. Medic. Assoc. Malawi* 14, 34-36.
- Middleton, E. (1998). Effect of Plant Flavonoids on Immune and Inflammatory Cell Function. In "Flavonoids in the Living System" (J. A. Manthey and B. S. Buslig, eds.), pp. 175-182. Springer US, Boston, MA.
- Nafiu, M. O., Abdulsalam, T. A., and Akanji, M. A. (2013). Phytochemical Analysis and Antimalarial Activity Aqueous Extract of *Lecaniodiscus cupanioides* Root. *J. Tropical Medic.* 2013, 4.
- Phillipson, J. D., and Wright, C. W. (1991). Antiprotozoal agents from plant sources. *Planta Med* 57, S53-9.
- Santos, R. F., Nunes, B. M., Sá, R. D., Soares, L. A. L., and Randau, K. P. (2016). Morpho-anatomical study of *Ageratum conyzoides*. *Revista Brasileira de Farmacognosia* 26, 679-687.
- Ukwe Chinwe V, Ekwunife Obinna I, Epueke Ebele A, and Ubaka Chukwuemeka M (2010). Antimalarial activity of *Ageratum conyzoides* in combination with chloroquine and artesunate. *Asian Pacific J. Trop. Medic.* 3, 943-947.