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Phytochemical screening with a Bioassay-Guided Toxicity and in Vivo Antimalarial Evaluation of Cleome gynandra Ethyl Acetate extract

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Abstract

The growing resistance to conventional antimalarial drugs necessitates the search for alternative therapies from natural sources. This study aimed to evaluate the antimalarial efficacy and toxicity profile of Cleome gynandra extracts, with emphasis on the ethyl acetate fraction. The aerial parts of C. gynandra were extracted sequentially using n-hexane, ethyl acetate, and methanol. The resulting extracts were screened for phytochemicals using standard methods and assessed for toxicity and in vivo antimalarial activity against Plasmodium berghei in Swiss albino mice. Toxicity was evaluated based on weight change, behavioral signs, and mean survival time (MST). Antimalarial activity was measured using parasitemia reduction. Among the three extracts, the ethyl acetate fraction demonstrated the highest safety margin and antimalarial efficacy, particularly at a dose of 100 mg/kg. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glucosides, phenols, and terpenoids. Treated mice exhibited significant reductions in parasitemia and prolonged survival times without signs of acute toxicity. The ethyl acetate extract of Cleome gynandra possesses potent antimalarial activity and favorable safety in vivo, supporting its traditional use and potential development as a natural antimalarial agent.

Keywords: Antimalarial activity, Cleome gynandra, Ethyl acetate extract, Phytochemicals, Plasmodium berghei, Toxicity

1. Introduction

Malaria remains a major global health challenge, particularly in sub-Saharan Africa, where resistance to conventional antimalarials continues to rise. There is an urgent need for alternative treatment strategies, including those derived from medicinal plants (World Health Organization, 2021; Okello et al., 2020). Cleome gynandra, also known as African spider plant, was selected for this study due to its long history of ethnomedicinal use across Africa and Asia. Traditionally, it is employed in the

treatment of inflammatory conditions, malaria, and gastrointestinal disorders. Scientific investigations have demonstrated that C. gynandra possesses antioxidant, antimicrobial, anti-inflammatory, and insecticidal properties, which may support its traditional application in disease treatment (Akinmoladun et al., 2019; Sharma & Patel, 2020). The plant is rich in flavonoids, terpenoids, and glucosinolates—compounds often associated with bioactivity against parasites. Its abundance in rural communities and safety in dietary use as a leafy vegetable further justified its investigation as a promising candidate for antimalarial drug development (Akinmoladun et al., 2019). This study aims to investigate the toxicity and in vivo antimalarial activity of sequential extracts of Cleome gynandra, with a focus on the ethyl acetate extract, through a bioassay-guided approach.

2. Literature review

Cleome gynandra is a widely distributed herbaceous plant valued for both its nutritional and medicinal properties. Native to Africa but now present across tropical and subtropical regions, it is semi-cultivated for its edible leaves rich in vitamins and minerals, particularly vitamin C, calcium, and iron (Chweya & Mnzava, 1997; Mishra et al., 2011). The plant is also used in traditional medicine to treat a variety of diseases, including diabetes, cardiovascular ailments, cancer, and rheumatism (Sabir & Aziz, 2015).

2.1. Description

Botanically, C. gynandra is characterized by glandular hairs, palmately compound leaves, and white to purple-tinged bisexual flowers (Mishra *et al.*, 2009). Phytochemical analyses reveal that the plant is rich in bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and terpenoids, contributing to its strong antioxidant and potential antidiabetic, anticancer, and antimicrobial properties (Linda & Anthony, 2015; Wanjala et al., 2020; Augustinus & Ritha, 2018; Shanmuganathan & Karthikeyan, 2013).

2.2. Phytochemistry of *Cleome gynandra*

Several studies confirm the plant's antioxidant potential through high levels of phenolics and flavonoids, particularly in its leaves (Linda & Anthony, 2015). Methanolic and ethanolic extracts have also demonstrated significant inhibitory effects on glucose diffusion and microbial activity (Naga et al., 2020; Abhilasha et al., 2021).

Given its bioactivity, *C. gynandra* is being studied as a potential source of antimalarial compounds. Antimalarial agents, derived from both synthetic and plant sources, target Plasmodium species, including *P. falciparum*, which causes severe malaria (Ashley et al., 2018; Mittra et al., 2013).

Table 1: Phytochemical screening of ethyl acetae extract of *Cleome gynandra*

Solvent	Extract code	Weight	Extract code
n-Hexane	CGH	36.41	CGH
Ethyl acetate	CGE	34.75	CGE
Methanol	CGM	62.79	GCM

Table 2: Phytochemical screening of ethyl acetae extract of *Cleome gynandra*

Phytochemicals	**Test**	**Inference**
Alkaloids	Mayer's Test	+
Saponins	Froth's Test	+
Taninns	Lead acetate's Test	+
Terpernoids	Salkowski's Test	+
Flavonoids	Alkaline Reagent's Test	+
Phenols	Ferric Chloride Test	+
Glucosides	Legal's Test	+

Table 3: Toxicity Study of *Cleome gynandra* Crude Extracts

Extract	Weight Changes (%)	Survival Time (MST in Days)	Toxicity Interpretation
Ethyl Acetate (100 mg/kg)	+9.72 (Gain)	18.67	Safe & least toxic. Recorded the highest weight gain highest Mean Survival Time (Days)
Methanol (100 mg/kg)	-2.05 (loss)	17.00	Toxic
n-Hexane (100 mg/kg)	+4.97 (Gain)	13.25	Toxic with the lowest survival times
Normal Saline (Control)	-	9.25	No antimalarial activity
Chloroquine (100 mg/kg)	+6.79 (Gain)	18.00	Safe, used as control

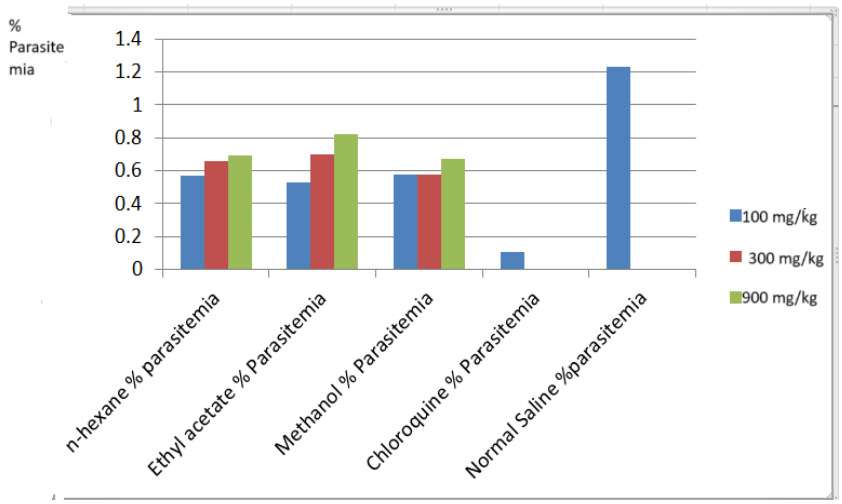

Figure 1: Effect of different doses of *Cleome gynandra* extracts on the Percentage Parasitemia of *P. berghei* in infected mice.

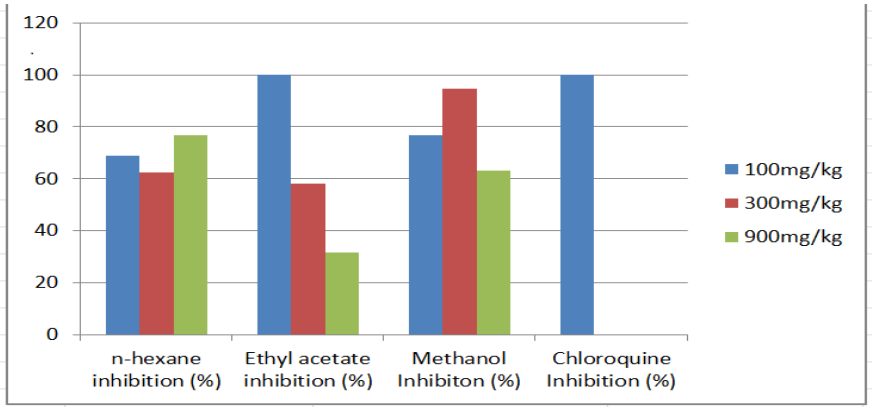

Figure 2: Percentage inhibition of *P. berghei* by different fractions of *Cleome gynandra* extracts

3. Research methodology

3.1. Materials

3.1.1. Plant Collection and Identification

Fresh Cleome gynandra plant's aerial part was collected at Idu Industrial Area of Abuja FCT by the Department of Botany and Forestry NIPRD, where a specimen was authenticated by Mr.Akeem A. Lateef, a taxonomist, in the herbarium with voucher specimen number NIPRD/H/7475.

3.2. Drying of plant materials

The plant sample was air-dried at room temperature and pulverized using a wooden manual mortar.

3.3. Extraction process from plant

The plant sample (the aerial part) was collected, air dried, and pulverized with a grinder. The powdered sample (3.10 kg) was macerated with 18.10 L of n-hexane for 72 hours with manual agitation at 3-hour intervals. The mixture was filtered, and the filtrate concentrated under reduced pressure using a rotary evaporator at 25 ℃ to recover the solvent. This procedure was repeated for ethyl acetate (18 L) extract and methanol (18 L) extract in order of increasing polarity. The extracts were then allowed to "air dry" by exposing the remaining solvent. Each of the extracts was coded as follows: n-hexane extract was coded CGH, ethyl acetate extract was coded CGE, and methanol extract was coded CGM. Each extract was weighed, and a little percentage of them was used for in vivo antimalarial and toxicity assays to ascertain the extract is the safest and most active among the three extracts.

3.4. Phytochemical screening

The ethyl acetate extract was screened for phytochemicals using standard Harboune 1998 protocols.

3.5. Antimalarial assay

3.5.1. Animals and parasite inoculation

Healthy albino mice (18–22 g) were acclimatized and inoculated intraperitoneally with *Plasmodium berghei* (1×107 infected red blood cells). Infected mice were left untreated for three days (D3) to allow parasitemia establishment.

The crude extracts from n-hexane, ethyl acetate, and methanol were screened for toxicity and in vivo antimalarial activity at the Department of Pharmacology and Toxicology, NIPRD, Abuja.

3.5.2. Animals and diet

All animals were handled according to the laboratory animal care guideline (CIOMS, 1985) and the recommended procedures of the Department of Pharmacology and Toxicology, National Institute for Pharmacology Research and Development. Healthy female albino mice (18 - 24 g) were used. They were housed under standard environmental conditions of temperature (26±2°C), relative humidity, and a 12-hour light/dark cycle. They were fed with standard rodent diet and allowed free access to water ad libitum. All experiments were performed in accordance with Standard Operating Procedures for studies involving whole animals.

3.5.3. Method of toxicology test

The toxicity of the Cleome gynandra extracts was assessed concurrently with the in vivo curative antimalarial assay using a non-invasive approach, as described by Ryley and Peters (1970). A total of 25 Swiss albino mice were inoculated intraperitoneally with the Plasmodium berghei ANKA strain and left untreated for three days to allow the infection to establish. On Day 3 post-infection, mice were weighed and randomly divided into five groups (n = 5 per group): a negative control group (normal saline), a positive control group (chloroquine, 100 mg/kg), and three extract treatment groups (n-hexane, ethyl acetate, and methanol extracts). Each extract was administered orally at doses of 100 mg/kg, 300 mg/kg, and 900 mg/kg once daily from Day 3 to Day 6. Treatment was done via oral gavage using sterile syringes and flexible feeding cannulae. Toxicity evaluation was based on non-invasive parameters, including: Percentage weight change: Mice were weighed on Days 3 and 7, and percentage weight gain or loss was calculated.

Mean survival time (MST): Mice were observed daily for 30 days post-treatment to assess survival, with the average survival time recorded for each group. Behavioral and physical signs: Mice were monitored for visible signs of toxicity such as lethargy, loss of appetite, abnormal posture, rough coat, reduced activity, and mortality. In this model, an extract producing the highest weight gain and longest mean survival time was interpreted as well tolerated and having low toxicity. This interpretation is based on the premise that during Plasmodium berghei infection, mice exhibiting stable or increased weight and prolonged survival generally indicate that the administered treatment is safe and non-toxic. Conversely, weight loss, early mortality, or abnormal behaviors suggest potential toxic effects.

3.5.4. Evaluation of curative activities of extracts (Rane's Test)

Evaluation of the curative potential of the extract against established infection was carried out as described by Ryley and Peters (1970). Twenty-Five (25) mice were inoculated with the Plasmodium berghei parasite and left untreated for three days (D3) post inoculation. The mice were then weighed and randomized into treatment groups of five mice each. Normal saline and Chloroquine were used as Negative and positive controls, respectively. The animals were treated for four days (D3-D6). On Days 3 and 7, each mouse was tail bled, and a thin blood film was made on a microscope slide. The films were stained with Giemsa stain and examined using a microscope at × 100 magnifications to

monitor the parasitaemia level. Each film was read 5 times. The average percentage parasitemia was determined, and the percentage inhibition for the extract was evaluated.

4. Results and discussions

4.1. Percentage yield of extract

The percentage yield of the various crude extracts is shown in Table 1

Methanol produced the highest extract with a weight of 62.79 g, followed by n-hexane with a weight of 36.41 g and ethyl acetate with a weight of 34.75 g. The methanol extract has the highest percentage yield, with a percentage yield of 2.02 %, followed by n-hexane with a percentage yield of 1.17 % and ethyl acetate with a percentage yield of 2.02 %. The ethyl acetate extract, being the safest and most active extract, was screened for phytochemicals in Table 2.

4.2. Phytochemical constituents

Phytochemical screening of ethyl acetate extract of Cleome gynandra is shown in Table 2

Preliminary phytochemical screening of the ethyl acetate extract revealed the presence of alkaloids, Saponins, flavonoids, tannins, terpernoids, phenols, and glycosides.

4.3. Non-invasive toxicity evaluation

Mice treated with ethyl acetate extract at 100 mg/kg exhibited weight gain and the highest MST compared to other groups (See Table 3). No signs of acute toxicity, such as tremors, convulsions, or mortality, were observed, suggesting good tolerability.

4.4. Assessment of extracts for non-invasive toxicity

Toxicological evaluation is a crucial step in drug discovery to determine the safety profile of bioactive compounds. The extracts were assessed for acute non-invasive toxicity in an animal model (mice) by monitoring signs of toxicity, including behavioral changes, weight loss or gain, and mortality, for a better understanding of the safety profile of the plant extracts, as shown in the Table. This approach allowed for the simultaneous observation of therapeutic efficacy and potential adverse effects of the plant extracts. Parameters such as body weight change, mean survival time (MST), and observable behavioral signs were monitored to infer safety. Extracts that produced significant weight gain and prolonged survival were considered well tolerated, while reductions in these parameters indicated possible toxicity. The findings from this integrated assessment are presented in Table 3.

4.5. Antimalarial activity

The ethyl acetate extract showed the highest percentage parasitemia (Figure 1) and inhibition (Figure 2) among the extracts, particularly at 100 mg/kg, with values approaching that of chloroquine. The order of activity was: ethyl acetate > methanol > n-hexane. The efficacy of *Cleome gynandra* Extracts on the Percentage Parasitemia of *P. berghei* in Infected Mice as shown in Figure 1.

The treatment with 100 mg/kg chloroquine (standard drug) resulted in the lowest parasitemia (0.11 %), confirming its known efficacy. Interestingly, a comparable reduction in parasitemia was observed with 100 mg/kg of ethyl acetate (0.53 %) and 100 mg/kg of n-hexane (0.47 %) fractions, suggesting that these extracts possess promising antiplasmodial activity.

The reduced level of parasitemia in groups treated with low doses (100 mg/kg) of ethyl acetate and n-hexane suggests a strong concentration-independent antimalarial response, indicating potential bioactive compounds in these fractions. In contrast, higher doses (300 mg/kg and 900 mg/kg) of these extracts showed a less pronounced suppression of parasitemia (ranging from 0.67 % to 0.82 %), possibly due to dose-dependent saturation, metabolic overload, or toxicity effects at higher concentrations which may interfere with the bioavailability or efficacy of the active constituents. The Inhibitory efficiency of *Cleome gynandra* Extracts against *Plasmodium berghei* in Infected Mice was shown in Figure 2.

The inhibitory activity of various solvent fractions of *Cleome gynandra* on *Plasmodium berghei* (as shown in the table in infected mice revealed a striking trend: the lower the administered dose (mg/kg), the higher the percentage inhibition. Notably, the 100 mg/kg ethyl acetate fraction achieved 100 % inhibition, equaling the antimalarial potency of the reference drug, chloroquine. This was followed by 300 mg/kg methanol (94.83 %) and 100 mg/kg methanol (81.90 %), while the lowest inhibition was observed in the 900 mg/kg ethyl acetate group (31.82 %).

The high activity of the extracts at lower doses may be attributed to optimal pharmacokinetic and pharmacodynamic interactions at those concentrations. At lower doses, the bioactive compounds are likely absorbed efficiently and distributed to the target sites without metabolic overload. In contrast, higher doses might lead to enzyme saturation, poor absorption, or induction of metabolic enzymes that accelerate the breakdown of active components, reducing their efficacy. Additionally, higher concentrations may cause antagonistic interactions among phytochemicals or trigger cytotoxic effects that impair host response to infection.

The significant antimalarial activity observed with the ethyl acetate extract of *Cleome gynandra* aligns with traditional claims of its therapeutic use. The presence of bioactive phytochemicals may contribute to its efficacy. The favorable toxicity profile suggests that the extract is safe at therapeutic doses.

5. Conclusion

This study demonstrates that the ethyl acetate extract of Cleome gynandra possesses potent and safe antimalarial properties. The presence of multiple phytochemical classes may be responsible for its efficacy. Compared to previous studies on herbal extracts with antimalarial potential, C. gynandra ethyl acetate extract stands out for its dual attributes of potency and safety. The curative model used effectively simulated clinical malaria, making the results more translatable. Further pharmacological and clinical investigations are warranted to explore its potential as an antimalarial drug candidate.

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