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Anti Malarial Activity of *Cassia Sieberiana* Leaf Extracts

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Abstract: This research was carried out to determine the antimalarial efficacy of *Cassia sieberiana* against *Plasmodium* parasites isolated from patients attending Nassarawa Specialist Hospital, Kano Nigeria. The research revealed that the leaf extracts of *C. sieberiana* are highly effective in killing *Plasmodium* parasite belonging to *falciparum* species. It was also established that the extracts are not harmful and are safe for human consumption. Accordingly, the study recommended pharmacological screenings with fractions of methanol extract as well as the isolation and identification of active compounds from the plant. The leaf extracts of *C. sieberiana* could be used for antiplasmodial therapy; however, it is imperative to carry out further tests to ascertain the effects on human organs such as heart and liver in order to determine the level of safety.

Keywords: Activity, *Cassia sieberiana,* Leaf, Extracts, *Plasmodium falciparum*.

1. INTRODUCTION

Malaria is a parasitic disease transmitted by the bites of female Anopheles mosquitoes infected with *Plasmodium* species, four of which infect humans; *P. falciparum* (the most deadly one), *P. vivax*, *P. malariae* and *P. ovale*. The disease primarily affects poor population in tropical and sub tropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites [1]. More than 40% of the world population is at risk of the disease [2]. An estimated 1.2 billion people are at high risk of transmission and half live in Africa [3].

In Nigeria, almost all malaria cases are caused by *P. falciparum* which is among the leading causes of death worldwide in 2004, from a single infectious agent. The search for new drugs based on plants is important due to the emergence and widespread of multiple drug-resistant malaria parasites, which require the development of new antimalarial drugs. Although vaccines could be the best long term control option, they are still undergoing clinical trials [4]. It is against the backdrop of the vast importance of *C. sieberiana* as a disease curing plant that it was considered necessary to experiment the effectiveness of the leaf extracts of the plant for antimalarial therapy.

1.1. Objectives of the Research

The main objectives of the study are to achieve the following:

a. To extract *C. sieberiana* leaf using different solvents.

b. To test the extracts for antiplasmodial activity.

c. To determine the effectiveness of *C. sieberiana* leaf extracts in killing *Plasmodium* parasites.

2. METHODOLOGY

2.1. Separation of the Erythrocytes

Blood sample with 5% parasitaemia collected from Nassarawa Specialist Hospital, Kano was centrifuged at 2500 r/m for 15 minutes. After centrifugation, the supernatant (plasma) was discarded while the sediments (erythrocytes) were further centrifuged with normal saline at 2500 r/m for 5 minutes. The supernatant was then discarded and the erythrocytes were suspended in normal saline.

2.2. Extraction

2.2.1. Collection of Plant Material

Fresh samples of *C. sieberiana* leaves for the study were collected from the Bayero University Kano (BUK) Botanical Garden. Identification was confirmed by the Botanist at the Biological Sciences Department, BUK, Nigeria. The voucher number of the specimen is 89.

2.2.2. Extraction

The aqueous and methanol extracts of the leaves of the plant were prepared according to the standard method described by Sofowora [5]. The plant samples were air dried and ground. The powdered materials (50g) were transferred into a soxhlet apparatus and then extracted exhaustively using solvents with varying polarity index, 95% benzene with polarity index 2.7 and 95% methanol with polarity index 5.1. Aqueous extract with polarity index 9.0 was obtained using percolation method and 5% H_2SO_4 was added to prevent fermentation. The extracts (benzene and methanol) were concentrated to dryness by evaporating the excess solvents at ambient temperature while aqueous extract was concentrated by heating in a water bath for about 15

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minutes and allowed to dry at ambient temperature. The residues obtained were transferred into pre-weighed sample containers and stored at 40 C before use.

2.3. Phytochemical Screening

The extracts of *C. sieberiana* were analyzed for the presence of secondary metabolites including: alkaloid, saponin, anthraquinone, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides according to standard method [6].

2.4. Preparation of Extracts

2.4.1. Preparation of the Test Concentrations

An electronic digital balance, model (FA2104A) Gulfex Medical and Scientific Company, England, was used to measure twenty milligram of each of the extracts and then dissolved in 1ml of DMSO in separate Bijou bottles (stock solution). Using serial doubling dilution, four different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of each extract were prepared.

2.4.2. Preparation of Culture Media (RPMI 1640)

The media was prepared by dissolving 10.4g of the powdered material into one litre of distilled water and then autoclaved at 121 degrees centigrade for 15 minutes as instructed by the manufacturers.

2.4.3. Antiplasmodial Assay

Equal volume of the extract solution (0.5ml) and the culture media were transferred into flat bottomed test tubes and labelled accordingly (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml). For each concentration of the extract, 0.1ml of the malaria positive erythrocytes was added and shaken gently to ensure even distribution of the erythrocytes. The test tubes were transferred into a bell jar containing burning candle. The cover of the bell jar was then replaced until the flame of the candle stopped burning. This supplied about 95% nitrogen, 2% oxygen and 3% carbon dioxide as described by Trager *et al*; [7]. The whole set up was transferred into an incubator maintained at 37^{o}C for 24 – 48 hrs. A control group consisting of culture media plus positive erythrocytes (negative control) and culture media plus positive erythrocytes and anti-malarial agent Athemeter (positive control) was incubated along with the test concentrations.

After 24 hrs of incubation, a thin smear from test tube was made on clean glass slides and fixed in absolute methanol then stained with Giemsa's stain. Each smear was observed under microscope using oil immersion to count the number of infected erythrocytes. The same procedure was repeated after 48 hours of incubation to determine the activity.

2.5. Determination of Activity

At the end of the incubation periods *i.e.* 24 and 48 hours, a drop of thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Giemsa's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination of the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after incubation period of 24 and 48 hours using the formula below:

% = N x 100

Nx

Where % = Percentage activity of the extracts

N = Total number of cleared RBC

Nx = Total number of parasitized RBC

RBC = Red Blood Cells [8].

2.6. Toxicity of *C. Sieberiana* Extracts Using Brine Shrimp Lethality Test

Toxicity test of the extracts was conducted to determine the level of lethal concentration of the plant fractions using brine shrimp eggs (Artemia salina leach).

2.6.1. Preparation of the Stock Solution

Methanol (2ml) was added to the fractions (0.02g) in a sterilized sample vials. The mixture was shaken until the test fractions dissolved completely in the solvent. 200µg /ml, 20µg /ml and 2µg /ml of the stock solution were each placed into a sample vial using micro syringe to prepare 1000µg /ml, 100µg /ml and 10µg /ml concentrations respectively. These were allowed to stay for about 24 hours in order to evaporate the solvent [9].

2.6.2. Hatching of the Brine Shrimp Eggs

Artemia salina (leach) eggs were added in a hatching chamber containing seawater. The hatching chamber was kept for 24-48 hours for the eggs to hatch into shrimp larvae [9].

2.6.3. Screening of Extract Fractions

A drop of DMSO and 4ml seawater were added to each of the 1000µg, 100µg and 10µg concentrations/ vials. Each dosage was tested in triplicate. Using dropper, 10 larvae of Artemia salina were introduced into each of the sample vials. Additional 0.9ml seawater was added to each sample vial in order to make the volume to 5ml. The larvae were allowed to stay in these vials for 24 hours after which the survivals were counted [10].

3. RESULTS

The result of the extraction showed that aqueous had higher extraction capacity on leaves of the plant than the other solvents while benzene had the least extraction capacity using both percolation and soxhlet methods (Table **1**).

Phytochemical screening of the plant extracts revealed the presence of several plant secondary metabolites that are responsible for antiplasmodial activity with more secondary metabolites observed in percolation extracts than soxhlet extracts as shown in Table **2**.

Determination of antiplasmodial activities of the extracts indicated that the extracts were very active against *Plasmodium* parasites (Table **3**).

The result of the brine shrimp experiment indicates that the Lethal Concentration (LC_{50}) is > 1000 µg/ml which

Table 1. Physical Properties of *C. Sieberiana* Extracts

Property	P (ME)	S (ME)	P (BE)	S (BE)	P (AE)	T (AE)
Weight of Plant Material (g)	50	50	50	50	100	100
Weight of Extract (g)	4.90	11.50	2.00	3.00	16.60	29.20
Percentage Yield (g)	9.80	23.00	4.00	6.00	16.60	29.20
Colour	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green
Texture	Gummy	Gummy	Oily	Oily	Crystalline	Crystalline

Key: P = Percolation, Soxhlet = Bark, (ME) = Methanol Extract, (BE) = Benzene Extract, (AE) = Aqueous Extract, T = Traditional.

Table 2. Phytochemical Constituents of *C. Sieberiana* Extracts

Phytochemical	P (ME)	S (ME)	P (BE)	S (BE)	P (AE)	T (AE)	P (AE)
Saponin	+	+	-	-	+	+	+
Tannin	+	+	-	-	+	+	+
Steroid	+	+	-	-	+	-	+
Amino acid	-	-	-	-	-	-	-
Anthraquinone	+	+	+	+	+	+	+
Flavonoid	+	+	-	-	+	-	+
Glycoside	+	+	+	+	+	-	+
Triterpenoid	+	+	+	+	+	+	+
Phytosterol	+	+	+	+	+	-	+
Alkaloid	-	-	-	-	-	+	-
Reducing sugar	+	+	+	+	+	+	+

Key: P = Percolation, S = Soxhlet, (ME) = Methanol Extract, (BE) = Benzene Extract, (AE) = Aqueous Extract, Traditional = T.

Table 3. Antiplasmodial Activity of *C. Sieberiana* Leaf Extracts

Extraction Method	Solvent	Conc mg/ml	Average No of Parasites/Field Before Incubation	Overall Average No of Parasites/Field At the End of Incubation	Percentage Elimination At the End of Incubation %
AT +ve Control		5.00	55	0.00	100
-ve Control		5.00	55	61.88	12.5*
Soxhlet	ME	1.25	50	0.00	100
		2.50		0.00	100
		5.00		0.00	100
		10.0		0.00	100
	BE	1.25	50	0.00	100
		2.50		0.00	100
		5.00		0.00	100
		10.0		0.00	100

Table 3. contd….

Extraction Method	Solvent	Conc mg/ml	Average No of Parasites/Field Before Incubation	Overall Average No of Parasites/Field At the End of Incubation	Percentage Elimination At the End of Incubation %
	AE	1.25	50	17.0	66.0
		2.50		12.3	75.4
		5.00		6.70	87.0
		10.0		4.00	92.0
Percolation	ME	1.25	55	0.00	100
		2.50		0.00	100
		5.00		0.00	100
		10.0		0.00	100
	BE	1.25	55	0.00	100
		2.50		0.00	100
		5.00		0.00	100
		10.0		0.00	100
	AE	1.25	55	7.00	87.0
		2.50		6.00	89.1
		5.00		14.0	74.5
		10.0		7.30	86.7

Key: AT = Athemeter, ME = Methanol, BE= Benzene and AE = Aqueous,

* Percentage of the parasite after incubation.

Table 4. Brine Shrimp Lethality Test

Concentration (µg/ml)	No of Shrimps that survived in *C.* Leaf Methanol extract
200	5
20	8
2	10

means that the *C.* leaf extracts are not harmful as indicated in Table **4** below:

4. DISCUSSION

Two methods, percolation and soxhlet were used in the leaf extraction process of *C. sieberiana*. However, the results obtained using both methods showed slight difference in the percentage yield of the extractions while there was no difference in the result of the secondary metabolites and antiplasmodial activity. The result of the extraction of the leaf indicated that in both the percolation and soxhlet methods, the aqueous yield is higher which may be related to the polarity index of the solvents with water having 9.0, followed by methanol with a polarity of 5.1 while benzene had the least yield with a polarity index of 2.7. Furthermore, the higher yield obtained in the soxhlet method could be attributed to the effectiveness and accuracy of the soxhlet machine. The results obtained from the extraction of *C. sieberiana* plant extracts are similar to the leaf extraction process of *C. occidetalis* using soxhlet machine where aqueous has the highest yield of 6.8%, followed by methanol which yielded 6%, petroleum ether 5.6%, chloroform 2.4% and benzene 1.6% [11].

The phytochemical screening of *C. sieberiana* leaf extracts showed the distribution of the presence of secondary metabolites in the extracts. The methanol leaf extract indicated presence of saponin, tannin, steroid, flavonoid, glycoside, triterpenoind, phytosterol, anthraquinone and reducing sugar. However, the benzene leaf extract showed presence of only glycoside, triterpenoid, phytosterol, anthraquinone and reducing sugar while aqueous contains saponin, tannin, triterpenoid, alkaloid and reducing sugar. These differences could be attributed to their polarity index.

The best antiplasmodial activity for the leaf extracts of *C. sieberiana* was obtained with methanol and benzene extracts. The extracts showed virtual absence of the parasite after 72

hours. This suggests that the activity of the extracts may be cytotoxic for *P. falciparum* thereby inhibiting their development. The aqueous leaf extract using percolation method showed 92% elimination at 10mg/ml, while the same concentration for aqueous traditional indicated 86.7% elimination after 72 hours. This indicates that the aqueous leaf extract is likely to be effective if the incubation period of the parasite is extended up to 7 days. However, the elimination rate using the percolation method was higher than the traditional method. This could possibly be due to the temperature generated from the boiling of the leaves in the traditional method.

Statistical analysis showed that there is no significant difference between soxhlet and percolation methods used, $P > 0.05$. However, there is a significant difference between the concentrations of the extracts and the hours of incubation, $P < 0.001$. There is also a significant difference between the concentrations and the Initial Parasitized Red Blood Cells (IPRBC), $P < 0.001$.

A related study on the antimalarial activity of Burkina Faso medicinal plants was carried out by Quattara *et al.*, [12]. The result indicated that the extracts of *S. madaga-scariensis, C. gluttinosum T. bakis* possess some measure of antimalarial activity. Methanol extracts showed higher activity than the aqueous extracts which are used in traditional medicine. The result of this study is similar to the result obtained using the *C. sieberiana* plant extracts, the focus of this research which also showed higher activity in methanol and benzene than aqueous.

A brine shrimp lethality test was carried out to ascertain the toxicity level of the *C. sieberiana* leaf extracts. The result of the experiment indicates that the Lethal Concentration (LC50) is > 1000 µg/ml which means, that the *C. sieberiana* plant extracts are not harmful. This further confirms that the leaf of *C. sieberiana* plant is safe for human consumption and could therefore be used for antiplasmodial therapy.

5. CONCLUSION

This study has revealed that the leaf extracts of *C. sieberiana* are highly effective in killing *Plasmodium* parasite belonging to falciparum species. This is a scientific basis for using *C. sieberiana* as an antimalarial plant. It was also established that the extracts are not harmful to humans. This indicates that the leaf extracts of *C. sieberiana* plant are safe for human consumption and could therefore be used for antiplasmodial therapy. However, it is essential to conduct pharmacological screenings with chemical fractions of methanol extract of *C. sieberiana* as well as the isolation and identification of active compounds from this plant. It is also imperative to carry out further tests to ascertain the effects of the leaf extracts on human organs such as heart and liver in order to determine the level of safety.

CONFLICT OF INTEREST

The author declares that there is no competing interest.

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