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Anti-Plasmodial Activity of the Ethanolic Stem-Bark Extract of *Anthocleista djalonenensis* on Chloroquine Sensitive Plasmodium

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ABSTRACT

Anti-plasmodial activity of the ethanolic stem-bark extract of *Anthocleista djalonenensis* on chloroquine sensitive Plasmodium berghei infection in mice was investigated in-vivo, to confirm its traditional use as an alternative malaria remedy. Phytochemical screening was carried out to identify the phytochemical constituents of the plant extract. The extract at various concentrations were evaluated for blood schizontocidal activity against Chloroquine-sensitive Plasmodium berghei infection in mice during early stage and established infections. The Lethal Dose (LD₅₀) of the extract was determined to be 2236mg/kg, while doses of 2500mg/kg/day and above were found to be lethal to the mice. *Anthocleista djalonenensis* extract administered (220, 440 and 660mg/kg/day) exhibited dose-dependent chemosuppression with a mean survival time comparable to that of the standard drug, Chloroquine (5mg/kg/day) at P <0.05, level of significannce. Evaluation of the schizontocidal activity of the plant extract at different concentrations as well as the standard drug (Chloroquine) were examined. The result indicated a dose-dependent chemosuppression of the parasitemia in early infection and a dose-dependent reduction in the parasitemia level during established infection stage, thus revealing the curative effect of the plant extract, justifying its wide spread use in herbal and traditional medicine, thus recommended for exploitation in malaria malarial therapy.

Keywords; Schizontocidal Activity, Chemosuppression, Phytochemical.

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INTRODUCTION

Malaria is a vector borne disease caused by protozoan parasites - *Plasmodium spp.* Each year it infects 515 million people and kills between 1 to 3 million people, the majority being children in the sub-saharan Africa [5]. In Nigeria malaria has been described as being holo-endermic with greater intensity in the wet seasons [2]. Most of the measures employed for the control of malaria have been based on traditional and crude measures; however they have been development of synthetic drugs for the treatment of the infection.

Most serious forms of the infection is caused by species such as *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovales* and *Plasmodium knowles*. Symptoms of Malaria includes fever, shivering, arthralgia, vomiting, and anemia, pains at joints. Infected people who live in regions where malaria is prevalent develop a limited immunity to the disease [13]. The cost of available anti-malarial drugs and their reduced effectiveness against malaria has led many Nigerian to use of some local herbs as remedies for the disease. Anti malarial herbal plants used in Akwa Ibom State of Nigeria includes, such plants as pawpaw (*Carica papaya*), lemon grass, bark of mango tree (*Mangifera indica*) and cabbage tree (*Anthocleista djalensis*).

Anthocleista djalensis is a medium size tree of the West Africa Tropical forest. The tree is about 10m tall and 12cm width. It has broad green leaves clustered at twig ends. Its outer bark is greyish and green below, It has light brown flowers and green smooth fruits [5]. According to [10] and [1], Its economic importance includes usage against sexually transmitted diseases particularly against activities of *Staphylococcus aureus* and *Escherichia coli*. As well as treatment of dysentery and other gastrointestinal diseases. In Mali. and in Cote D'voire, it has been used as poison antidote against leprosy, oedema, elephantiasis of the scrotum, infertility, chest pain, kidney diuretics and dysmenoria and pulmonary anomalies. Authors reporting on the geographical distribution of *Anthocleista djalensis* indicates that, the plant is found abundantly in areas of West Africa viz: Benin, Cameroon, Burkina Faso, Ghana, Niger, Togo and Nigeria [3,4,5,7,9].

The existing gap is that *Anthocleista djalensis* has been used by most traditional healers for treatment of malaria but has not had a recorded investigation of its anti-plasmodial activities in Akwa Ibom State of Nigeria. The study was aimed at investigating the anti-plasmodial activities of stem-bark of the plant as it has been used as the mainstay of the malaria treatment of malaria in traditional practices in Nigeria. We therefore evaluate the blood schizontocidal activities of the ethanolic stem-bark extract of *Anthocleista djalensis* against *Plasmodium berghei* in mice.

The specific objectives of the study were therefore, to determine the phytochemical constitution of the stem-bark of *Anthocleista djalensis* and to investigate the anti-plasmodial activities of stem-bark of the plant. The results obtained from this investigation provided information on the phytochemical constituents of the stem-bark of *Anthocleista djalensis*.

MATERIALS AND METHODS

Plant Material Collection and Extraction

The stem-bark of *Anthocleista djalensis* (specimen) were collected from a forest environment in Nsit Atai Local Government Area of Akwa Ibom State of Nigeria. The specimens were washed with clean water, air dried for seven days and powdered. It weighed about 1.75kg. It was exhaustively macerated in ethanol to dryness in a water bath at 40°C. The dried extract were put into a beaker, sealed with aluminum foil and stored in refrigerator. The dried ethanolic extract was dissolved in normal saline to make the stock solution from which the various doses administered to the mice were prepaid.

Animals

Swiss albino mice (14-24g) of either sex were obtained from the University of Uyo Animal Houses in Nigeria. The animals were fed with standard pelleted feed, kept in standard cages and acclimatized for 10days before being used for the experiment.

Pytochemical screening

Pytochemical screening of the extract was carried out employing standard procedures [8,14].

Malaria Parasite Inoculation

The Chloroquine-sensitive strain of *Plasmodium berghei* was obtained from the National Institute of Medical Research in Nigeria, and maintained by sub passage in mice. The inoculums consisted of 5×10^7 *Plasmodium berghei* parasitized red blood cells per ml. This was prepared by determining both the percentage parasitemia and red blood cells count of the donour mouse and diluting the blood with isotonic

saline in proportions indicated by both determinations. Each infected blood contained about 1×10^7 *Plasmodium berghei* parasitized red blood cells.

Drug Administration

A feeding cannula was used to treat the infected mice orally with the control drug Chloroquine (CQ) and the extract.

Evaluation of Schizontocidal Activity on Early Infection (4-Day Test)

The schizontocidal activity of the extract was evaluated using the method described by Knight and Peters (1980). Each mouse was inoculated on the first day (day 0) intraperitoneally (under the skin) with 0.2ml of infected blood containing about 1×10^7 *Plasmodium berghei* parasitized erythrocytes. The animals were divided into five groups of five mice each group and were orally administered with different doses, 220, 440, 660 mg/kg/day doses of *Anthocleista djalonensis* stem-bark extract, Chloroquine 5mg/kg/day for the positive control groups and an equivalent volume of distilled water (negative control group) for four consecutive days (day 0 to day 3). Between the hours after the administration of the last dose, thin blood films were made from the tail blood and stained with Giemsa stain and the percentage parasitemia was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as:

$$\text{Average percentage chemosuppression} = 100 [(A-B)/A] \quad (1)$$

Where, A is the average percentage parasitemia in the negative control group and B is the parasitemia in the test group.

Evaluation of Schizontocidal Activity on Established Infection (Curative or Rane Test)

The evaluation of the curative potential of the extract was done using a method similar to that described by [12]. The mice were injected intraperitoneally with a standard inoculum of 1×10^7 *Plasmodium berghei* infected erythrocytes on the first day (day 0). Seventy-two hours later (day 3), the mice were divided into five groups of three mice each. The groups were orally administered with different doses (220, 440 and 660 mg/kg/day) was given to the positive control groups. The extract was dissolved in normal saline and given once daily for five days (D3-D7). Thin films stained with Giemsa stains were prepared from the tail blood of each mouse daily for five days to monitor the parasitemia. The mean survival time (MST) for each group was determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 30 days (day 0-29). The Parasitemia levels of the animals that survived after 30 days of monitoring were determined using blood thin films made from the tail of each surviving animal. t-test was used for data analysis at $P < 0.05$ significance level.

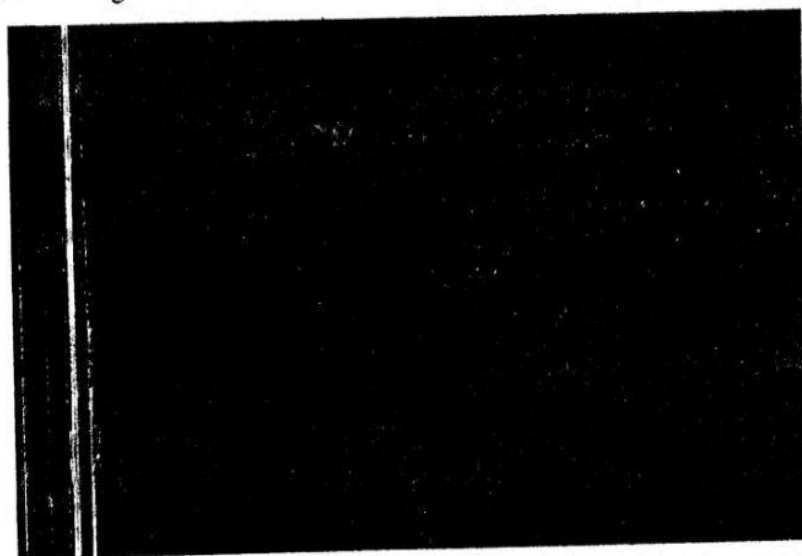


Fig.-1: Picture of *Anthocleista djalonensis*

RESULTS AND DISCUSSION

Phytochemical Screening Results

Table 2 shows the results of Phytochemical screening of the ethanolic stem-bark extract of *Anthocleista djalonensis* revealed the presence of compounds including saponins, tannins, cardiac glycosides, flavonoids, deoxy-sugar and terpenes.

Schizontocidal Activity during Early Infection

The stem-bark of *A. djalonensis* produced a dose-dependent chemo-suppressive effect when tested against early infection with daily doses of 220, 440, 660mg/kg administered orally causing a significant ($P < 0.05$) Chemo-suppression (Table II) which are significant when compared to the control group. The standard drug, chloroquine caused significant chemosuppression than that of the extract-treated groups.

Schizontocidal Activity During Established Infection (Curative Test)

On established infection it was observed that there was a dose-dependent reduction in the parasitemia of the extract-treated groups, while the control group showed a daily increase in parasitemia. Chloroquine (5mg/kg) also produced a marked daily reduction in parasitemia. The percentage parasitemia of the extract-treated groups on day 7 as well as that of the control and chloroquine-treated group is shown in table II. The mean survival time (MST) of the mice in various groups were 12.3, 16.3, 21.3, 32.0 and 14.0 days for 220, 440, 660 mg/kg/day of extracts, 5mg/kg/day of chloroquine and 0.2ml per day of the control groups respectively. The animals that survived in the extract treated groups as well as the chloroquine-treated group was found to be parasite-free.

The stem-bark extract had been found to contain saponins, tannins, cardiac glycosides, flavonoids, deoxy-sugar and terpenoids. These compounds may be responsible for the anti-plasmodial activity of the extract, either singly or in synergy, though the main active components had not been identified. Saponins, alkaloids and flavonoids had been reported to be responsible for the anti-plasmodial activities in plants [11] and [6]. The presence of these compounds has elicited the observed anti-plasmodial activity, either singly or in synergy. However, the results of the phytochemical screening indicated the presence of alkaloids in the stem-bark. This observation was in consonance with that of [4] who stated that the leaves and the stem-bark contains alkaloids.

The result also indicates that stem-bark extract possesses blood schizontocidal activity as evident from the chemosuppression obtained during the four days test. A significant ($P < 0.05$) activity was also recorded during the established infection, which was comparable to the standard drug, Chloroquine (5mg/kg/day). The ethanolic stem-bark extract of *Anthocleista djalonensis* was screened phytochemically to identify the bioactive components which characterize its activities against *Plasmodium berghei* infection in mice. Stock solution of the extract was prepared and various concentrations redrawn to make various doses during four-day and curative tests. The tail-blood films were prepared and average percentage parasitemia and suppression determined.

Table- 1: Identification and Classification of *Anthocleista djalonensis*.

English Name	Botanical Name	Specific Epithet	Genus	Family	Order	Class
Cabbage Tree	<i>Anthocleista Djalonensis</i>	<i>djalonensis</i>	<i>Anthocleista</i>	<i>Loganiaceae</i>	<i>Gentianales</i>	<i>Magnoliopsida</i>

Table -2: Phytochemical screening of stem-bark of *Anthocleista djalonensis*

Phytochemical Contents	Types of test
Test for Tannins	Bromine Water Test

	Ferric Chloride Test
Test for Phlobatannins	Using Fomaldehyde + Dilute Hydrochloric acid + Potassium Hydroxide
Test for Saponins	Frothing Test
	Sodium Carbonate and Fehling's Solution Test
Test for Anthraquinones	Borntrager's Test
Test for Cardiac Glycoside	Salkowski Test
	Lieberman's Test
Test for Flavonoids	Shinoda Reduction Test
Test for Alkanoids	Dragendorff's Reagent Test
	Mayer's Reagent Test
	Picric Acid (Hager's Reagent) Test
	Wagner Reagent Test
Test for Terpenes	Using Acetic Anhydride + Concentrated Sulphuric Acid
Test for Deoxy-Sugars	Glacial Acetic Acid + Ferric Chloride + Sulphuric Acid

Table -3: Phytochemical Qualitative Analysis of the Ethanolic Stem-bark of *Anthocleista djalensis*

Test	Observation	Inference
Tannins	No colour change.	-
(a) Bromine Water Test	Blue-black precipitate formed.	++
(b) Ferric Chloride Test		
Phlobannins Formaldehyde and Hydrochloric Acid Test	No precipitate.	-
Saponins	Persistent frothing formed.	++
(a) Frothing Test	Reddish-brown precipitate formed.	++
(b) Sodium Carbonate and Fehling's Solution Test		
Anthraquinones (Borntrager's test)	No colour change.	-
Cardiac Glycosides	Reddish-brown colour was observed at the interphase.	++
(a) Salkowski Test	Colour change from violet to blue.	++
(b) Lieberman's Test		
Flavonoids Shinoda Reduction Test	A foamy-orange colour was observed.	+++
Alkanoids	No colour change observed.	+
(a) Mayer's Reagent Test	Yellow colour was Observed.	-
(c) Picric Acid (Hager's Reagent) Test		
Deoxy-sugars Glacial Acetic Acid+Ferric Chloride+Sulphuric Acid	A pink colouration was observed.	++
Terpenes Using Acetic	A separation was formed with a violet ring at the lower layer.	+++

Anhydride+Concentrated Sulphuric Acid		
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Where: +++ Present in high concentration; ++ Moderately present; + Slightly present; - Absent

Table- 4: Anti-plasmodial Activity of *Anthocleista djalensis* During Four-Days Test.

Drug/Extract	Dose (mg/kg/day)	Average percentage(%) parasitemia	Average percentage(%) chemo-suppression
A. djalensis	220	21.67 ± 2.08	22.61
A. djalensis	440	20.32 ± 1.53	27.39
A. djalensis	660	18.67 ± 1.70	33.32
Chloroquine	5	4.67 ± 1.53	81.37
Control	0.2ml	28.80 ± 1.00	-

Table-5: The Mean Survival Time of Mice Receiving Various Doses of A. djalensis Stem-bark Extract, Chloroquine and Normal Saline (control).

Drug/Extract	Dose (mg/kg/day)	Mean Survival Time (day)
A. djalensis	220	12.33 ± 1.15
A. djalensis	440	16.33 ± 2.08
A. djalensis	660	21.33 ± 0.58
Chloroquine	5	32.00 ± 1.73
Control	0.2ml	14.00 ± 1.00

CONCLUSION

The study has revealed that the ethanolic stem-bark extract of *Anthocleista djalensis* possesses anti-plasmodial activity as shown in its ability to suppress *Plasmodium berghei* infection in mice in all the evaluations. These evaluations have justified the traditional use of the plant by traditional healers for the treatment of malaria. The study has shown that stem-bark of *Anthocleista djalensis* contains some bioactive components that have enhanced its activity against *Plasmodium berghei* infection. Attempts should be made to isolate, identify and characterize the active compounds and explore their anti-malarial effects and toxicity to animals as to encourage its usage as anti-malarial drugs in clinical medicine.

REFERENCES

1. Adiaratou, T., Drissa, D., Seydou, D., Hilde, B., Smestad, P., Ethnopharmacological Ethnomedicine Journal, **7** (2005) 1.
2. Bruce-chwatt, L. J. British Medical Journal, **286** (1983) (2376) 1457.
3. Burkill, H. M., Royal Botanic Gardens. Kew Journal, **2** (1995) 1.
4. Burkill, H. M., Royal Botanic Gardens. Kew. Journal, **2** (1999) 1.
5. Chev., Bulletin Soc. Bot. France, **4**. (2005) 47(4):1908.
6. Christensen, S. B., Kharazmi, A., 2001. In: Tringail, C. (Ed.), Taylor and Francis, London. (2001) 379.
7. Etukudo, I. The Verdict Press, Uyo, AKS (2003)76.
8. Harbone, J. B., 1983. A guide to modern techniques of plant analysis. New York. Chapman and Hall. New York, pp. 482-495.

9. Igoli, J. O., Tor-Anyiin, T.A and Igoli, N.P., African Journal of Traditional, Complementary and Alternative Medicines, **2** (2005) 134.
10. Okoli, A.S., Iroebu, C. U., Journal of Ethnopharmacology, **92** (2005) 135.
11. Philipson, J. D., Wright, C. W., Planta Medica, **57** (1991) 553.
12. Ryley, J., Peters, W., American Journal of tropical Medical Parasitology, **84** (1990) 209.
13. Smith, A. H.. In Microsoft Encarta Encyclopaedia (Premium Suit, 2006).
14. Trease, A., Evans, W. C. (1989) Pharmacognosy 13th ed. Bailliere Tindal Londonpp 688.

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Theresa M. Udofia was born in December 1961. She is a lecturer in the department of Science Education in the university of Uyo. She has several publications in journals and books. She has attended ten (10) conferences and workshops. Mrs. Udofia has won awards including the Best Conference Paper Award by Science Teachers Association of Nigeria (STAN), 2006. Her research interest areas are chemistry Education.