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Isovitexin, the main phytoconstituent of *Anthocleista djalonensis,* may be responsible for the plant's antimalarial properties

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Abstract

Several studies have reported on the antimalarial properties of *Anthoclestia djalonensis.* This study was therefore conducted to validate this report and identify the phytocomponents of the plant that might be responsible for its antimalarial activity. The crude methanol extract of the plant was fractionated into ethyl acetate and *n*-butanol fractions using the liquid-liquid fractionation method. After a 5-day curative study, the plant's crude extract and fractions at 200 mg/kg showed varying levels of antimalarial properties, with the *n*-butanol fraction showing the highest parasite clearance (86.8%), followed by the crude extract (77.3%), and the ethyl acetate fraction (49.32%). HPLC analysis of the ethyl acetate and n-butanol fractions revealed isovitexin and its derivative as the plant's major phytoconstituents, and these compounds may be responsible for the plant's antimalarial property.

Keywords: Anthocleista djalonensis; Isovitexin; HPLC; Plasmodium; Malaria

1. Introduction

Herbal medicines have played an important role in the discovery and development of antimalarial drugs. This is especially true given that well-known antimalarial drugs such as quinine and artemisinin were discovered in plants. It is possible that through research into herbal remedies used in folkloric malaria treatment, new antimalarial compounds may be discovered (1,2).

Several species of *Anthocleista* (family Gentianaceae), including *A. djalonensis, A. vogelii, A. nobilis, A. grandiflora, A. schweinfurthii,* and *A. liebrechtsiana*, are commonly found in tropical African countries, including Nigeria, and have been used ethnomedicinally for the treatment of malarial fever and other disease conditions such as typhoid, hypertension, abdominal pain, hemorrhoids, syphilis, diabetes, and as a contraceptive and laxative.(3–6) The stem, root bark, and leaves of *A. djalonensis* have traditionally been used to treat malaria in Nigeria, and scientific research has confirmed the plant's antimalarial activity.(7–10).

With the goal of finding new drug compounds that are active against the malarial parasite, this study was conducted to validate the antimalarial activity of *A. djalonensis* and to identify the plant's phytoconstituents that might be responsible for its antimalarial activity.

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2. Materials and Methods

2.1. Animals and Housing

Male Swiss albino mice of various weights were used in this study. The animals were obtained from the animal facility of the Department of Pharmacology and Toxicology at Nnamdi Azikiwe University in Nigeria. The mice were housed in a clean and well-ventilated cage and had free access to food and water.

2.2. The Parasite: Plasmodium berghei

Plasmodium berghei-infected donor mice were obtained from the animal facility at the University of Nigeria, Nsukka. A volume of 0.2 ml standard inoculum of 10^6 parasitized erythrocytes from a donor mouse was used to infect the test animals intraperitoneally.

2.3. Sample Collection, Extraction, and Fractionation

Fresh leaves of *A. djalonensis* were collected from Umuoji Village in Anambra State, Nigeria. The plant leaves were thoroughly washed and air-dried at room temperature (25 °C). After drying, the leaves were pulverized using a mechanical grinder. A 2.5 g weight of the powdered leaves was cold macerated in methanol (3 L) for 48 h. The obtained extract was sieved using a muslin cloth and further filtered with Whatman No. 1 filter paper. The resulting filtrate was then concentrated using a rotary evaporator at a reduced temperature and pressure. Using the liquid-liquid fractionation method, the crude plant extract was then fractionated with ethyl acetate and *n*-butanol.

2.4. High Performance Liquid Chromatography (HPLC)

Following the method described by Eze et al. (2019), about 2 mg of each of the plant's ethyl acetate and *n*-butanol fractions were reconstituted with 2 ml of HPLC-grade methanol. The mixtures were sonicated for 10 min and thereafter centrifuged at 3000 rpm for 5 min. A volume of 100 µL of the dissolved samples was transferred into HPLC vials containing 400 µL of HPLC grade methanol. HPLC analysis of the samples was carried out using a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germing, Germany). Detection was at 235, 254, 280, and 340 nm. The separation column (125 x 4mm: length x internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. The compounds were detected by comparing the retention times and UV spectra with the in-built library.

2.5. *In-vivo* Anti-malarial Assay

The antiplasmodial effect of *A. djalonensis* was evaluated using a 5-day curative study described by Afolabi and Oyewole (2020).(11) A total of 25 mice were used for the study, and the animals were grouped into 5 groups of 5 mice per group. All the animals were inoculated with *P. berghei* by intraperitoneal administration of 0.2 ml of diluted blood sample from a donor mouse such that it contained approximately $1x10^6$ of the infected red cells. The parasite was allowed to multiply in the host's body within a 72 h incubation period following the infection. A thin film was then made from blood samples retrieved from the tail region of each animal and fixed with ethanol, after which it was stained with 10% Giemsa stain and examined under a 100x objective lens for parasitemia (pre-treatment parasitemia). The samples were then administered orally to the animals, with each group receiving one of the treatments listed in Table 1. Five days post-administration, thin blood films were made with blood from the tail vein of the mice. These were fixed, stained, and examined under a 100x objective lens for parasitemia (post-treatment parasitemia). Percentage (%) parasite reduction was calculated using the formula: $a - b/a \times 100$ where a = pre-treatment parasitemia, b = post-treatment parasitemia.

3. Results and Discussion

The results of the *in vivo* antimalarial evaluation of *A. djalonensis* confirm the antiplasmodial property of the plant. Based on the basal parasitemia (pre-treatment) and the level of parasite clearance post-treatment, the plant's crude extract and fractions, as well as the positive control (artemether-lumefantrine), reduced the parasite burden of the *Plasmodium*-infected mice.

Table 1 Result of *In-vivo* antimalarial assay of *A. djalonensis*

Treatment groups	Pre-treatment	Post-treatment	% Clearance
Group 1 [negative control (10 mL/kg distilled water)]	16.5 ±0.66	17.75 ± 2.32	-7.6
Group 2 [positive control (0.24 mg/kg ACT)]	15.3 ±0.95	5.42 ± 1.06*	64.5
Group 3 [crude extract (200 mg/kg)]	23.30 ±1.88	5.29 ±0.66**	77.3
Group 4 [ethyl acetate fraction (200 mg/kg)]	20.72± 0.26	10.5± 1.88**	49.32
Group 5 [*n*-butanol fraction (200 mg/kg)]	46.04 ±4.10	12.68 ±1.39**	86.8

*P<0.05, **P<0.01 when compared with control (distilled water).

The methanol crude extract and fractions of the plant showed significant (P<0.05) reduction in the parasite load after treatment of the *P. berghei* – infected mice; with the *n*-butanol fraction showing the highest parasite clearance (86.8%), followed by the crude extract (77.3%), and the ethyl acetate fraction (49.32%).

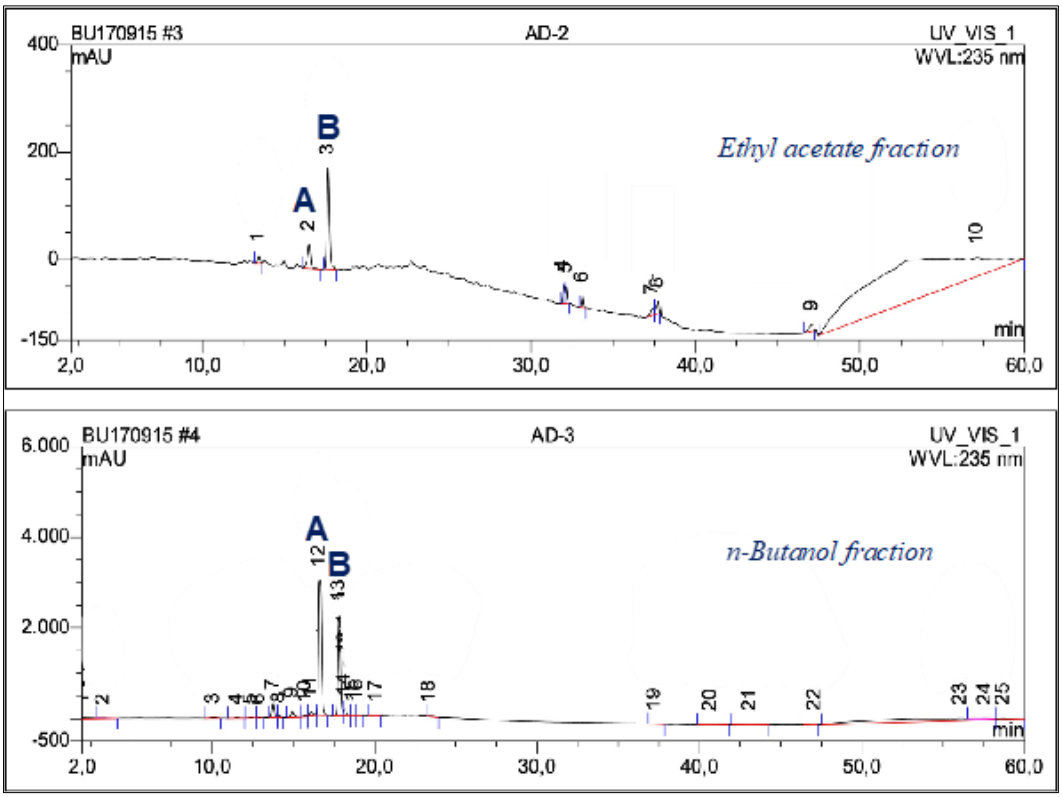

Figure 1 HPLC Chromatograms Showing the Abundance of Isovitexin and Its Derivative in the Ethyl Acetate and *n*-Butanol Fractions of *A. djalonensis.* The detection of an isovitexin derivative (A) is indicated by peaks 2 and 12 of the ethyl acetate and n-butanol fractions, respectively; while isovitexin (B) is indicated by peaks 3 and 13 of the ethyl acetate and n-butanol fractions, respectively

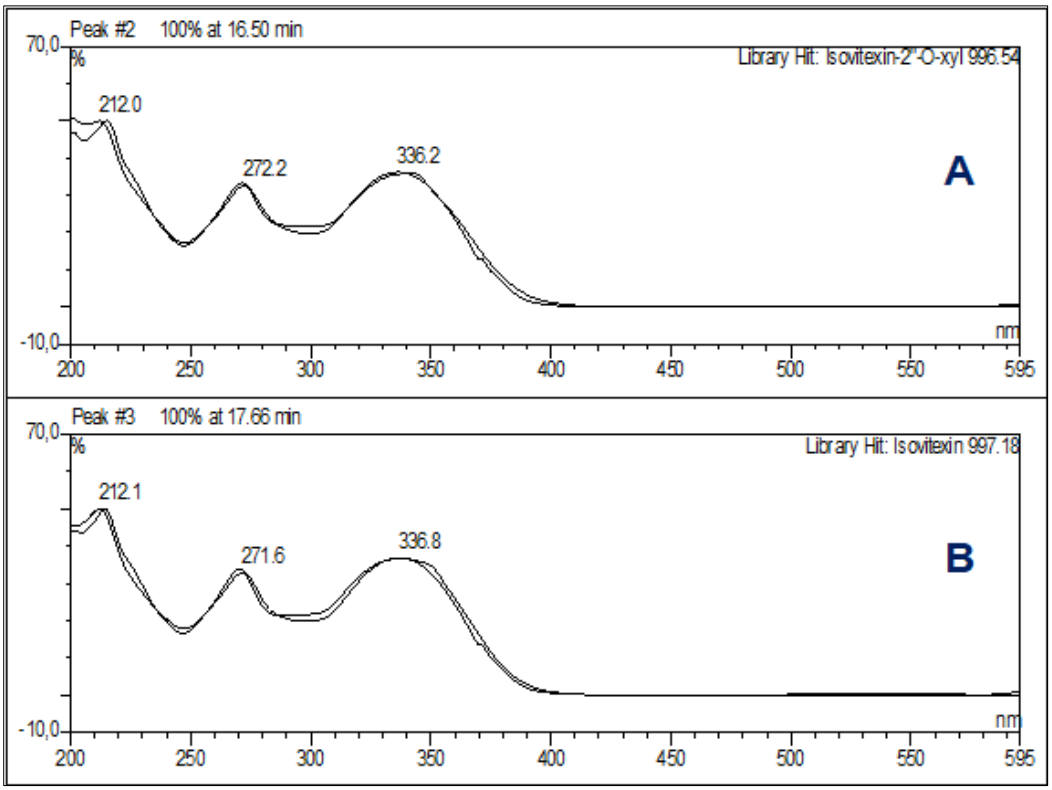

Figure 2 UV spectra of an Isovitexin Derivative (A) and Isovitexin (B)

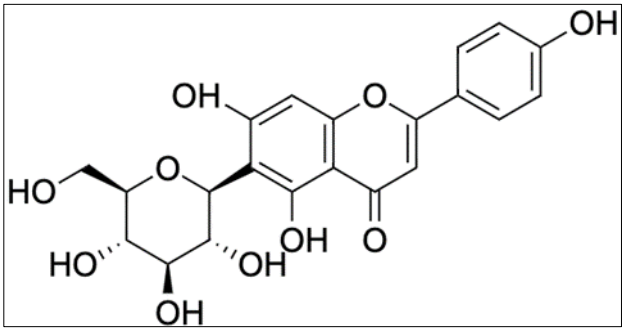

Figure 3 Structure of Isovitexin ($C_{21}H_{20}O_{10}$, 432.1051 g/mol)

Malaria is an acute febrile illness caused by *Plasmodium* parasites, which are spread to people through the bites of infected female *Anopheles* mosquitoes. Of the five *Plasmodium* species that cause malaria in humans, *P. falciparum* and *P. vivax* pose the greatest threat. *P. falciparum* causes the most severe and deadly form of malaria and is most prevalent on the African continent.(12)

According to the latest World Malaria Report(13), there were an estimated 247 million cases of malaria in 84 malaria-endemic countries worldwide in 2021, an increase from 245 million in 2020, with most of this increase coming from countries in the WHO African Region. Children under 5 accounted for about 80% of all malaria deaths in the Region. Nigeria accounted for the highest percentage of global malaria deaths (31.3%), followed by the Democratic Republic of the Congo (12.6%), the United Republic of Tanzania (4.1%), and Niger (3.9%). These four African countries accounted for just over half of all malaria deaths worldwide.(12,13)

For uncomplicated *P. falciparum* malaria, artemisinin-based combination therapies (ACTs) remain the best available treatment. However, on a global scale, plasmodial resistance to artemisinin has been identified. Given the high reliance on ACTs in Africa, complete treatment failure due to antimalarial drug resistance could have very serious

consequences.(14) Finding new malaria drugs appears to be an important approach to averting the threat of the disease and the development of drug resistance to the few existing drugs.

The results of this study, as well as those of several other studies(7,9,10,15), confirm the antiplasmodial activity of *A. djalonensis* extracts and the plant's potential as a probable source of new bioactive compounds for the development of new antimalarials. The antiplasmodial activity of *A. djalonensis* can be attributed to the individual and synergistic actions of its numerous bioactive phytochemical constituents.

HPLC analysis of the ethyl acetate and n-butanol fractions of *A. djalonensis* revealed the presence of two major compounds, isovitexin and its derivative (Figures 1-3), which may be responsible for the plant's anti-plasmodial activity.

Isovitexin (also known as homovitexin or saponaretin) is a naturally occurring bioactive glycosyl flavonoid constituent of many medicinal plants (16). Isovitexin and its parent compound vitexin are both derivatives of apigenin, a well-known flavone with antimalarial properties that have been reported in several studies.(17–21) Flavonoids are a large family of polyphenolic compounds and one of the most common secondary metabolites found in plants. This group of compounds has been found to possess antimalarial activity and a variety of other biological activities.(18,21–24)

Aside from its antiplasmodial properties, isovitexin has been reported to have a variety of biological properties such as antitumor, anti-diabetic, anti-inflammatory, and antioxidant.(25–29) Isovitexin and its derivatives were also detected as major compounds in another species of *Anthocleista* (Ngwoke et al., 2018). Isovitexin and its derivative have been identified in many plants with known antimalarial properties, including *Artemisia annua* (30), *Ficus Sansibarica* (31), *Ormocarpum kirkii* (32), *Prosopis* species (33), and *Cannabis sativa* (24).

Using molecular docking protocols, David et al. (2019)(24) reported the high potential of isovitexin as an antimalarial compound with possible inhibition of *P. falciparum* dihydrofolate reductase. Isovitexin, followed by its parent compound vitexin, displayed higher binding affinity and lower free energy than the positive controls pyrimethamine and cycloguanil, which are known antimalarial compounds.

4. Conclusion

The results of this study showed that *A. djalonensis* possesses considerable antiplasmodial activity, confirming its use in folk medicine for the treatment of malaria and associated symptoms. The plant's crude extract and fractions showed varying levels of antimalarial properties. HPLC analysis of the ethyl acetate and *n*-butanol fractions revealed isovitexin and its derivative as the plant's major phytoconstituents, and these compounds may be responsible for the plant's antimalarial property.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest related to this article.

Statement of ethical approval

The authors hereby declare that the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed in the study, and that the experimental protocols were reviewed and approved by the ethics committee of Nnamdi Azikiwe University, Nigeria.

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