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Research Article

A Study on *Justicia flava* (Forssk.) Vahl.: Pharmacognostic Characterization and Antiplasmodial and Anti-Inflammatory Properties of the Leaves

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Justicia flava (Acanthaceae) is utilized in folk medicine for treating malaria, inflammatory conditions, and respiratory infections. In this study, the antiplasmodial and anti-inflammatory activities of the 70% hydroalcoholic leaf extract of *J. flava* (JFE) were investigated. The antiplasmodial activity was assessed by the suppressive test and Rane's curative test. The carrageenan-induced paw oedema assay in chicks was used to assess the anti-inflammatory activity. In Rane's curative test, JFE significantly suppressed the parasitaemia level ($p < 0.0001$) by 56.85%, 63.85%, and 77.85% at 30, 100, and 300 $mg{\cdot}kg^{-1}$, respectively. The highest suppression of parasitaemia in the suppressive test was 51.31% at 300 $mg{\cdot}kg^{-1}$. The extract prolonged the survival time of infected mice significantly and prevented weight loss and hypothermia which are cardinal signs of *P. berghei*-induced infection. JFE inhibited foot oedema in chicks with a maximum percentage inhibition of $54.00 \pm 5.20\%$. Pharmacognostic characterization of *J. flava* revealed it to have simple, petiolate, oppositely arranged leaves which are broadly lanceolate in shape with entire to slightly crenate margins. Microscopy of the leaf showed wavy-walled epidermal cells, diallelocytic stomata, abundant clothing and secretory trichomes, pitted vessels, collateral conjoint vascular bundles, cystoliths, and prismatic calcium oxalate crystals. Flavonoids, tannins, phytosterols, triterpenoids, coumarins, alkaloids, and saponins were detected in the plant. This study has provided important parameters for the correct identification of *J. flava* and given the scientific credence to its antimalarial and anti-inflammatory effects in traditional medicine. *J. flava* is a potential source of new antimalarial and anti-inflammatory compounds.

1. Introduction

The genus *Justicia*, with over 600 species of scandent perennial herbs and shrubs, represents the largest genus in the family Acanthaceae [1]. Its species are usually found in warm and temperate regions of the world including Africa, Asia, and America. *Justicia* spp. are recognized for numerous ethnomedicinal applications including their use for treating

inflammatory diseases, diabetes, fever, malaria, asthma attacks, respiratory tract infections, bacterial infections, and sexually transmitted diseases [1, 2]. *Justicia* species have demonstrated significant bioactivities making them important sources of therapeutic agents. These include antiviral [3], antimicrobial [4], antioxidant [5], anticancer [6], anti-inflammatory [7], hepatoprotective [8], antidiabetes [9], anticonvulsant, sedative, and anxiolytic [10] properties. The antimalarial activity of several *Justicia* species has also been well reported [11]. *J. betonica*, *J. carnea*, *J. adhatoda, J. schimperiana*, and *J. flava* are species used to treat malaria in Uganda, Cameroon, Ethiopia, Kenya, Nigeria, and Ghana [11–14]. In a previous study, the leaves of *J. schimperiana* demonstrated significant prophylactic and curative effects against *Plasmodium* infection in mice [15]. The leaves of *J. carnea* also demonstrated significant *in vitro* antiplasmodial effects against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* strains [11]. *J. carnea* leaf extract was also found to exert a modulatory effect on the antiplasmodial activity of chloroquine and artesunate [16]. In another study, extracts of the leaves of *J. betonica* and *J. adhatoda* caused a significant suppression of *P. falciparum* schizont growth in an *in vitro* assay [17, 18].

Justicia flava is one of the most widespread species of the genus in West Africa [19]. It is a perennial herb or shrublet, which grows up to about 450 mm high, and is characterized by bright yellow terminal inflorescences, embedded in leaf-like bracts. The plant is called "*afama*" or "*ntumunum*" in the local Ghanaian Akan language and is used to treat malaria, fever, yaws, dysentery, and diarrhoea [20, 21]. Other ethnomedicinal uses of the plant across the African continent include vermifuge, emetic, analgesic, wound healing, against respiratory tract infections, pulmonary diseases, menstrual disorders, miscarriages, oedema, gout, paralysis, epilepsy, and convulsions [21–23]. Though several medicinal uses have been reported for *J. flava* in traditional medicine, only few scientific investigations have been carried out to substantiate the biological effects of the plant. These include its wound healing [20], antioxidant [24], antimicrobial [20], tocolytic [22], analgesic, and antipyretic [23] activities. *J. flava* is well known for its use in relieving fever and joint aches associated with malaria. Meanwhile, there is currently no scientific report which gives credence to its antimalarial effect. In continuing our studies on the potential of the Ghanaian flora as valuable sources of bioactive agents, this study reports the antimalarial and anti-inflammatory effects of the leaves of *J. flava.* This is to give scientific credence to its use in folk medicine for the treatment of malaria and inflammatory conditions. Important macromorphological and micromorphological features of *J. flava* are also described.

2. Materials and Methods

2.1. Plant Material Collection and Extraction. J. flava leaves were harvested from Abetifi in the eastern part of Ghana in March 2022. Sample authentication was done by Dr. George Henry Sam, Herbal Medicine Department, KNUST, and a specimen with voucher number KNUST/HMI/2014/L084 was deposited at the herbarium. The leaf samples were carefully washed with clean tap water, air-dried under shade for a week, and ground into powder using a mechanical grinder. Extraction was carried out by cold macerating approximately 1000 g of the powdered leaves in 70% ethanol for 72 h. The filtrate was reduced to a green syrupy extract on a rotary evaporator and further dried in an oven (40°C). A semisolid dried extract (JFE) weighing ~87 g (percentage yield = 8.7%w/w) was obtained and stored in an airtight container till needed for use.

2.2. Pharmacognostic Studies

2.2.1. Macroscopic and Microscopic Assessment. The leaves of *J. flava* were examined for organoleptic characteristics including taste, colour, odour, and texture. Macroscopic features of the leaf such as the leaf type, leaf arrangement, and lamina characteristics were noted. Microscopic assessment was carried out with the aid of a light microscope (DM-700) with a camera attached. Free-hand sections of the cleared leaf lamina, midrib, and petiole were observed under the microscope, and photomicrographs of cell types and cell inclusions were taken at ×10 and ×40 magnifications. The epidermal number, stomatal number and index, palisade ratio, veinlet termination, and vein-islet numbers were calculated as previously described [25].

2.3. Phytochemical Screening. Major classes of secondary metabolites were screened for, following previously described protocols [26].

2.4. Biological Activity Screening

2.4.1. Animals. Cockerels (*Gallus gallus*, strain Shaver 579) were obtained from Akropong farms (Kumasi, Ghana) one-day after hatch and transferred to the vivarium of the Department of Pharmacology, KNUST, Kumasi. The chicks were housed in groups of 12 chicks per cage in stainless steel cages ($34 \times 57 \times 40\ cm^2$). They were maintained at room temperature of 29°C under overhead incandescent illumination granting a 12 h light-dark cycle. The chicks were fed with chick mash (GAFCO, Tema, Ghana) and water. The chicks were used for experiments at 7 days of age.

Swiss albino mice (16–28 g) and chloroquine-sensitive *Plasmodium berghei* (ANKA strain)-infected donor albino rats were obtained from the Animal Facility Centre (AFC) of the Noguchi Memorial Institute for Medical Research (NMIMR), Legon, Accra, Ghana. The animals were kept in a vivarium at the Pharmacology Department, KNUST, where all experiments were carried out. They were housed in cages with wood shaven as bedding under a 12-hour natural light-dark cycle. The temperature of the lab was maintained at 27 ± 1°C with a relative humidity of 60–70%. The animals were fed with rodent feed and supplied with water *ad libitum*. All procedures employed were permitted by the Animal Research Ethics Committee (AREC) of KNUST. All experimental animals were handled according to the instructions given by the National Institute of Health

Guidelines for the Care and Use of Laboratory Animals (2011).

2.4.2. Animal Grouping and Dosing. For all *in vivo* assays, animals were put into five groups consisting of five mice each. These included three test groups which received doses of the test extract orally, the vehicle-treated group, which was given normal saline, and the positive control group which was administered the standard drug.

2.4.3. Acute Oral Toxicity Assay. The acute oral toxicity assay of JFE extract was performed according to the fixed dose method as given by the Guideline 420 of the Organization of Economic Cooperation and Development (OECD) [27]. Accordingly, Swiss albino mice, either male or female, were selected randomly and placed in two groups of five animals each and fasted overnight. Group 1 received a dose of JFE 5000 $mg \cdot kg^{-1}$ bodyweight (solubilized in distilled water), and group 2 received 0.2 mL of normal saline. The general behaviour/well-being of mice as well as any signs of toxicity (including change in skin colour, salivation, diarrhoea, lacrimation, change in fur, nostril discharge, lethargy, convulsion, tremor, and death) were observed continuously for an hour, then subsequently every 30 min for 4 h, then after 24 h, and daily for the next 14 days. If three or more mice survived, the LD_{50} was stated to be more than 5000 $mg \cdot kg^{-1}$.

2.5. In Vivo Antiplasmodial Activity

2.5.1. Inoculation of Parasite. Healthy mice were infected with the *Plasmodium* parasite by intraperitoneal injection of 0.2 mL of inoculum (infected blood with the parasitaemia level: 1×10^7 parasitized erythrocytes) as described by Baah et al. [28].

2.5.2. The 4-Day Suppressive Test (Prophylactic Assessment). The method described by Baah et al. [28] was followed in this assay. Briefly, mice infected with the *Plasmodium* parasite were selected randomly and grouped into five groups (5 mice/group). Three hours after parasite inoculation, the treatment groups received JFE 300, 100, and 30 $mg \cdot kg^{-1} \cdot day^{-1}$ (*p.o*); the negative controls received normal saline, 0.2 mL (*p.o.*); and the positive controls were given artesunate, 4 $mg \cdot kg^{-1} \cdot day^{-1}$ (*i.p.*). Treatment of mice was done at the same time each day and continued for the next four days (day 0 to day 3). After treatment on day 3, blood samples were obtained from the tails of mice in all treatment groups for analysis of parasitized RBCs. This was done without euthanizing the animals. Thin blood smears were fixed on glass slides with absolute MeOH, stained permanently with 10% Giemsa solution, and viewed under a light microscope at an objective magnification of ×100. Infected RBCs were counted from 5 randomly selected fields of view, and the percentage parasitaemia was determined as follows:

$$\%\ \text{parasitaemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100. \quad (1)$$

Percentage parasitaemia suppression was calculated as

$$\%\ \text{parasitaemia suppression} = \left[\frac{A - B}{A}\right] \times 100. \quad (2)$$

Here, A is the average % parasitaemia in the untreated group and B is the average % parasitaemia of the treated groups.

2.5.3. Rane's Curative Assay. This assay investigated the curative potential of JFE following a previously described method by Baah et al. [28]. Infection of mice was performed as described in Section 2.5.1. Three days after infection, the mice were grouped into five groups (5 mice/group). Groups 1, 2, and 3 (treatment groups) received JFE 30, 100, and 300 $mg \cdot kg^{-1} \cdot day^{-1}$ (*p.o*), respectively, group 4 received 0.2 mL of normal saline orally, and group 5 received artesunate, 4 $mg \cdot kg^{-1} \cdot day^{-1}$ (*i.p.*). Treatment continued for the next four days (day 3 to day 7). After treatment on day 3 and day 7, blood samples were obtained from the tails of mice in all treatment groups for analysis of parasitized RBCs. Thin blood smears were fixed on glass slides with absolute MeOH, stained permanently with 10% Giemsa solution, and viewed under a light microscope at an objective magnification of ×100. The level of parasitaemia on days 3 and 7 was determined by the formulas in Section 2.5.2.

2.5.4. Monitoring Survival Time. The length of survival time (in days) for each mouse was recorded within a 30-day period of observation in both suppressive and curative assays. The mice were observed from the day of infection to the day of death (i.e., if death occurs). The mean survival time (MST) was then determined by the formula:

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Sum of mice in the group}}. \quad (3)$$

2.5.5. Assessment of Body Weight and Body Temperature. The body weights of animals were taken on a digital balance and the rectal temperatures were taken with a digital thermometer. In the suppressive assay, these parameters were recorded on the day of infection (day 0) and after treatment on the 3rd day. In the curative assay, these parameters were taken on the 3rd and 7th days of treatment.

2.6. Carrageenan-Induced Footpad Oedema Assay in Chicks. The anti-inflammatory assay was performed as previously described [29]. Foot pad oedema was induced by injecting the subplantar tissue of the right footpad of chicks with 10 μL of 2% carrageenan solution. Before the induction of oedema, the footpad diameter was measured. Exactly 1 hour after carrageenan injection, the chicks were treated with JFE

extract suspended in normal saline at doses of 300, 100, and 30 mg·kg^{-1} given orally. Diclofenac sodium (10, 30, and 100 mg·kg^{-1}*i.p*) was given as a reference drug and normal saline (2 mL, *p.o.*) as the negative control. The diameter of the chick footpad was recorded hourly for 5 hours with the aid of vernier callipers. The raw footpad diameters were used to determine the percentage change in foot pad size as follows:

$$\%\ \text{change in foot pad size} = \frac{(\text{ft} - \text{fo})}{\text{fo}} \times 100. \quad (4)$$

Here, f_o is the foot pad diameter at time 0 and f_t is the foot pad diameter at time "*t*."

2.7. Data Management Analysis. The results of the experiments were analysed using GraphPad Prism (version 8 for windows, San Diego, USA). The data are given as the mean ± standard error mean (SEM). One-way analysis of variance (ANOVA) was used to make comparisons between the untreated and treated groups. Dunnet's *post hoc* test was used for multiple comparisons between tests.

3. Results

3.1. Organoleptic and Macromorphological Characteristics. The organoleptic and macromorphological examination of *J. flava* leaves revealed that *J. flava* has simple, green, petiolate leaves which are oppositely arranged on the stem (Figures 1(a) and 1(b)). The leaves are lanceolate to broadly lanceolate with entire to slightly crenate margins, arcuate venation, and a slightly rough surface (Figures 1(c) and 1(d)). The plant bears terminal flowers subtended in leafy bracts. The powdered leaf sample has a bitter taste and nonspecific odour. A summary of the organoleptic and macromorphological features of *J. flava* leaf is provided in Table 1.

3.2. Microscopic Characteristics of J. flava Leaf

3.2.1. Leaf Lamina. Microscopy of the cleared leaf laminar displayed wavy-walled anticlinal epidermal cells on both surfaces. Diacytic stomata, i.e., stomatal pores with two subsidiary cells positioned perpendicular to the guard cells having common walls obliquely positions in a C-shape (diallelocytic stomata), were abundant. Glandular trichomes with a multicellular head and a unicellular stalk were also observed. Vein-islets (Vi) and veinlet terminations (Vt) were observed (Figures 2(a)–2(d)).

3.2.2. Transverse Section (T/S) of the Midrib and Petiole. Microscopy of the T/S of the midrib showed a planoconvex-shaped midrib with one layer of polygonal-shaped epidermal cells externally covered by a thin cuticle. Several uniseriate multicellular clothing trichomes were found on the upper epidermal layer and few on the lower epidermis. About 4-5 layers of closely packed collenchyma were observed above the lower epidermis and below the upper epidermal layer. Several parenchyma cells filled the midregion, at the centre of which collateral conjoint vascular bundles in the open arch were located. Two smaller meristeles were observed in the upper region placed beneath the palisades on either side, slightly above the central meristele.

The T/S of the petiole displayed a circular-shaped petiole with undulating outline having abundant multicellular uniseriate nonglandular (covering) trichomes as well as glandular trichomes on the outer surface. A thin layer of cuticle, followed by one layer of polygonal epidermal cells and collenchyma, was observed. Parenchyma filled the core. Vascular bundles were present as meristeles, radially arranged along the periphery of the petiole. Xylem was located toward the interior and phloem to the exterior (Figures 2(e)–2(j)).

Microscopy of the leaf powder displayed prismatic calcium oxalate crystals, pitted xylem vessels, and lignified fibres (Figure 3). Leaf surface constants are presented in Table 2.

3.3. Phytochemical Screening. Major classes of plant secondary metabolites including tannins, glycosides, triterpenoids, phytosterols, alkaloids, coumarins, and flavonoids were identified in the leaves of *J. flava* (Table 3).

3.4. Acute Toxicity. The hydroalcoholic leaf extract of *J. flava* at an oral dose of 5000 mg·kg^{-1} did not result in any behavioural, neurological, or physical changes such as staggering gait, anxiety, sedation, agitation, emesis, excessive salivation, straub tail, or diarrhoea in mice during 24 hours of monitoring and for 14 days after treatment. No death was recorded. The LD_{50} was estimated to be more than 5000 mg kg^{-1}.

3.5. In Vivo Antiplasmodial Activity of J. flava Leaf Extract

3.5.1. Four-Day Suppressive Test. The suppressive test was employed to assess the ability of *J. flava* leaf extract (JFE) to suppress parasitaemia. JFE demonstrated a significant ($p < 0.0001$) dose-dependent inhibition of parasite growth at the doses tested when compared to the untreated group. The most remarkable parasitaemia suppression (51.31%) was demonstrated by JFE 300 mg·kg^{-1}. The extract also significantly prolonged the mean survival rate of mice. JFE 300 mg·kg^{-1} gave the longest survival time of 23.85 (±1.50) days. The positive control, artesunate, however, produced a higher suppression of parasitaemia (92.14%) than all doses of the leaf extract. The results are presented in Table 4.

3.5.2. Effect of J. flava on Body Temperature and Body Weight in the Suppressive Test. All treated and untreated mice infected with *P. berghei* experienced a decrease in body temperature from the day of infection till treatment was completed. Nevertheless, the change in rectal temperature recorded in infected mice in the negative control group was greater than that of the JFE and artesunate-treated groups (Table 5). Treatment with JFE also prevented a decline in the body weight of *P. berghei*-infected mice and rather caused

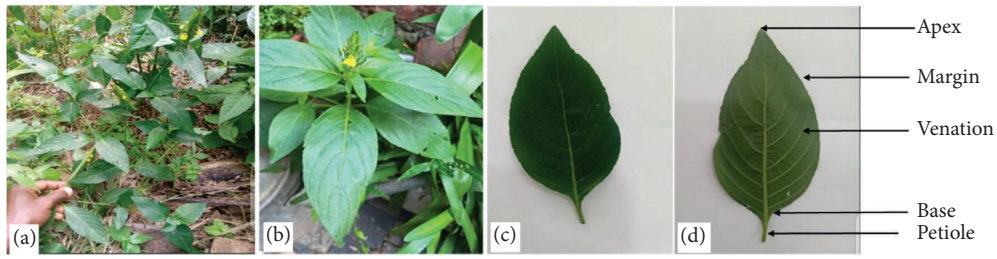

Figure 1: (a) *J. flava* plant in its natural habitat; (b) oppositely arranged simple leaves on a stem; (c) upper surface; (d) lower surface.

Table 1: Organoleptic and macromorphological characteristics of *J. flava* leaves.

Parameter	Description
Taste	Bitter
Odour	Nonspecific
Colour	
Upper surface	Deep green
Lower surface	Light green
Leaf type	Simple, petiolate
Leaf arrangement	Opposite
Shape	Broadly lanceolate
Margin	Slightly crenate
Apex	Broadly acute
Base	Attenuate
Venation	Arcuate
Length	7–12 cm
Width	2.5–5 cm
Thickness	—
Surface	Slightly rough

a slight increase in weight of mice. Contrary to this, the normal saline-treated group experienced a decrease in weight after the induction of parasitaemia (Figure 4).

3.5.3. Rane's (Curative) Test. After an established infection on day 3, all doses of JFE extract administered (30, 100, and 300 $mg{\cdot}kg^{-1}$) caused a remarkable decrease in the parasitaemia level by day 7. The effect was significant and dependent on the dose administered ($p < 0.0001$). The highest dose of JFE, 300 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$, remarkably suppressed parasitaemia by 77.85% by day 7. Artesunate (4 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$) exhibited a higher curative ability with a percentage parasitaemia suppression of 92.48% on day 7. The duration of survival of infected mice treated with JFE and artesunate was also substantially prolonged at all doses contrary to the untreated group (Table 6).

3.5.4. Effect of J. flava on Body Weight and Body Temperature in the Curative Test. All doses of *J. flava* averted loss of weight in infected mice between days 3 and 7 ($p < 0.0001$). The saline-treated group, however, experienced a substantial loss in weight by day 7 (Figure 5). Treatment with artesunate also prevented weight loss in infected mice. A slight reduction in temperature was observed for all *P. berghei*-infected mice from the 3rd to the 7th day (Table 7). The change in body temperature for JFE- and artesunate-treated mice was, however, less pronounced compared to the temperature change in the negative control group.

3.6. Effect of J. flava Leaf Extract on Carrageenan-Induced Footpad Oedema. Subcutaneous injection of 2% carrageenan into the chicks' footpads caused moderate acute inflammation observed as foot oedema. Figures 6(a) and 6(c) represent the time course curves, showing the progression of inflammation in treated and untreated groups, observed over a 6 h period. Figures 6(b) and 6(d) illustrate the total calculated area under the curve for each treatment group, which depicts the total foot oedema that occurred over the 6 h period of the experiment. For the treated groups, i.e., JFE- and diclofenac-treated groups, an increase in oedema was produced between the 1st and 2nd hour after which a sharp decrease in foot pad volume was recorded from the 3rd to the 6th hour. The percentage footpad oedema reduction in chicks treated with JFE was dependent on the dose-dependent and significant ($p < 0.0001$) when compared to the negative control group. The most remarkable inhibition of oedema was $54.04 \pm 2.52\%$, produced by JFE 300 $mg{\cdot}kg^{-1}$ (Table 8). The reference drug, diclofenac, also significantly ($p < 0.0001$) reduced oedema by $73.88 \pm 5.33\%$ dose at 100 $mg{\cdot}kg^{-1}$. In the negative control group, however, footpad oedema heightened between the 2nd and 3rd hour and slightly decreased from the 4th hour with substantial oedema present even at the 6th hour.

4. Discussion

In this study, the antiplasmodial and anti-inflammatory activities of the 70% hydroalcoholic extract of *J. flava* leaves were studied. The LD_{50} of *J. flava* leaf extract (JFE) was determined to be more than 5000 $mg{\cdot}kg^{-1}$ based on the results of the acute oral toxicity test. This report is congruent with the report by Bafor et al. who also reported an LD_{50} above 5000 $mg{\cdot}kg^{-1}$ for *J. flava* leaf extract [30].

The antiplasmodial activity of JFE was investigated by the suppressive and curative tests [31]. In both assays, the antiplasmodial effect is based on the percentage suppression of parasitaemia and the average survival period of infected mice in the treated group relative to the untreated group. *J. flava* leaf extract demonstrated the potential to reduce parasitaemia in both early (~17–52%) and late or established malaria infection (~57–71%). The extract demonstrated a better curative ability than suppression, suggesting that the extract may be a better option for treating an established

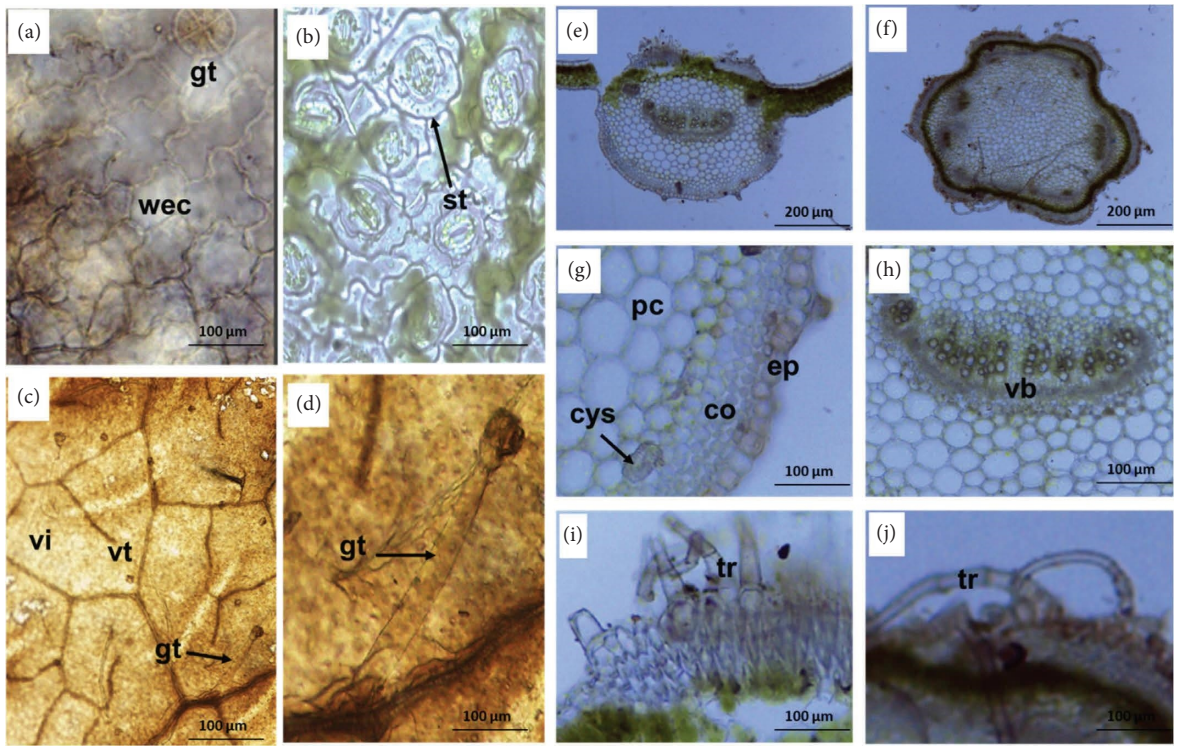

Figure 2: (a) Leaf laminar showing wavy-walled epidermal cells (wec) and multicellular head of glandular trichome (gt); (b) diacytic (diallelocytic) stomata (st); (c) vein-islets (vi), veinlet termination (vt), and glandular trichome (gt); (d) glandular trichome (gt) with unicellular stalk and multicellular head (×40); (e, f) T/S of the midrib and petiole (×10); (g) T/S of midrib showing epidermal cells (ep), collenchyma (co), parenchyma (pc), and cystolith (cys); (h) open arch vascular bundle (vb); (i) multicellular nonglandular trichomes (tr) on the outer surface of midrib; (j) multicellular nonglandular trichomes (tr) on the outer surface of petiole.

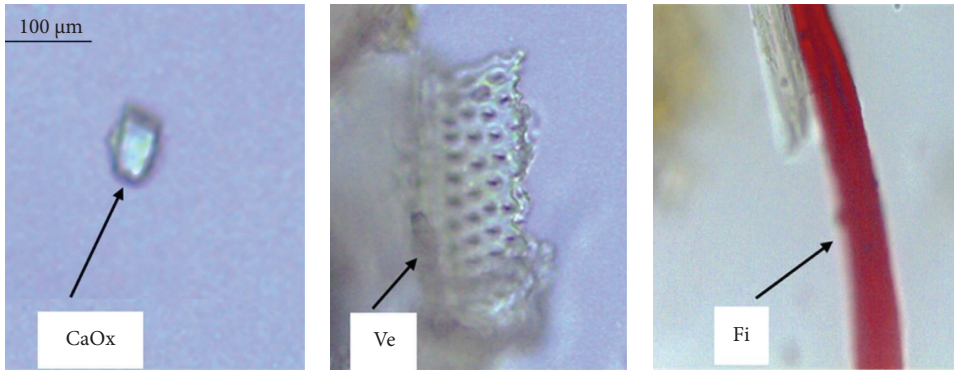

Figure 3: Microscopy of powdered leaf (×40) ((a) CaOx-prismatic calcium oxalate crystal; (b) Ve-pitted xylem vessel; (c) Fi-lignified fibre).

Table 2: Leaf surface constants of *J. flava*.

Parameter	Result
Palisade ratio	5.5 ± 0.14
Stomatal number (mm^2)	18–(20)–23
Stomatal index (%)	19.5
Vein-islet number (mm^2)	3.77
Vein termination number (mm^2)	4.02

Values are presented as a mean ± SEM ($n = 5$).

Table 3: Preliminary phytochemical screening of *J. flava* leaves.

Secondary metabolite	Test	Result
Tannins	Ferric chloride test	+
Glycosides (general)	Fehling's test	+
Alkaloids	Dragendorff's test	+
Saponins	Frothing test	+
Triterpenoids	Salkowski test	+
Phytosterols	Liebermann Burchard's	+
Flavonoids	Alkaline reagent test	+
Coumarins	Fluorescence test	+

+: detected.

Table 4: Level of the parasitaemia level, % suppression, and mean survival time of the *P. berghei*-infected mice treated with JFE in the suppressive test.

Sample	Dose (mg kg^{-1})	% parasitaemia	% suppression	Mean survival time (days)
NC	2 mL/kg	66.58 ± 1.66	—	12.80 ± 0.46
JFE	30	55.13 ± 0.25*	17.20	17.00 ± 0.38*
JFE	100	47.84 ± 0.49**	22.15	19.62 ± 0.62*
JFE	300	32.42 ± 1.63****	51.31	23.85 ± 0.50**
ART	4	5.23 ± 0.09****	92.14	29.20 ± 0.58**

Values are presented as the mean ± SEM, $n = 5$; NC = vehicle-treated group; ART = artesunate. Values are significantly different at $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{****}p < 0.0001$ compared to the negative control group.

Table 5: The effect of *J. flava* on the rectal temperature in the 4-day suppressive assay.

Sample	Dose (mg kg^{-1})	Rectal temperature		
		Day 0	Day 4	Δ temperature
NC	2 mL	37.8 ± 0.33	36.03 ± 1.21	−1.77
JFE	30	37.04 ± 0.36	36.78 ± 0.45	−0.26
JFE	100	36.56 ± 1.01	36.41 ± 1.06	−0.15
JFE	300	36.94 ± 0.97	36.56 ± 0.23	−0.38
ART	4	37.24 ± 0.11	37.18 ± 0.56	−0.06

Values presented as the mean ± SEM, $n = 5$; NC: negative control; ART: artesunate; Δ temperature = relative change in rectal temperature.

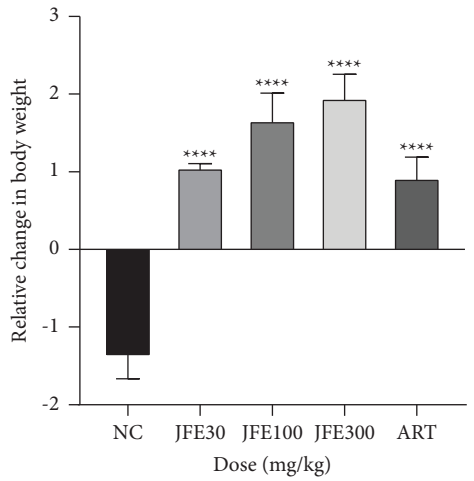

Figure 4: Relative change in body weight of *P. berghei*-infected mice in the 4-day suppressive test. Values are presented as the mean ± SEM, $n = 5$. Values are significantly different at $^{****}p < 0.0001$, compared to the vehicle-treated group. NC: negative control, JFE: *J. flava* leaf extract, and ART: artesunate 4 mg·kg^{-1}.

Table 6: Level of the parasitaemia, % suppression, and mean survival time of *P. berghei*-infected mice in Rane's curative test.

Sample	Dose (mg·kg^{-1})	% parasitaemia		% suppression	Mean survival time (days)
		Day 3	Day 7		
NC	2 mL	60.39 ± 3.07	69.52 ± 1.60	—	11.23 ± 0.23
JFE	30	69.19 ± 1.78	30.00 ± 1.94***	56.85	19.00 ± 0.55
JFE	100	67.99 ± 2.75	25.13 ± 1.33****	63.85	21.00 ± 0.56
JFE	300	70.98 ± 3.72	15.40 ± 1.11****	77.85	24.00 ± 0.17**
ART	4	63.41 ± 2.72	5.23 ± 0.94****	92.48	30.00 ± 0.00***

Values are presented as the mean ± SEM, $n = 5$; NC = vehicle-treated group; ART = artesunate. Values are significantly different at ** $p < 0.005$, *** $p < 0.001$, and **** $p < 0.0001$ compared to the negative control group.

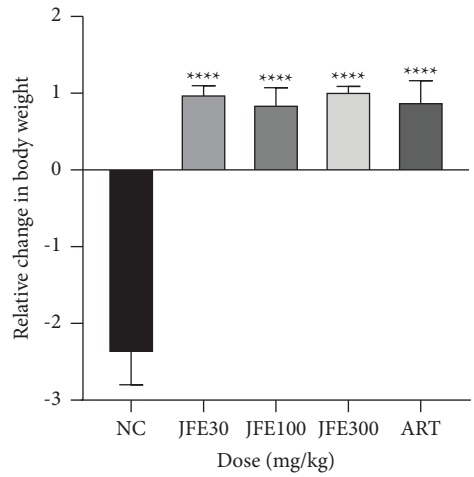

Figure 5: Relative change in body weight of *P. berghei*-infected mice in the curative test. Values are presented as the mean ± SEM, $n = 5$. Values are significantly different at **** $p < 0.0001$, compared to the vehicle-treated group. NC: negative control, JFE: *J. flava* leaf extract, and ART: artesunate 4 mg·kg^{-1}.

Table 7: The effect of *J. flava* on the rectal temperature in the curative test.

Sample	Dose (mg·kg^{-1})	Rectal temperature		Δ temperature
		Day 3	Day 7	
NC	2 mL·kg^{-1}	36.80 ± 5.21	36.18 ± 4.13	−0.62
JFE	30	37.04 ± 3.11	37.00 ± 1.97	−0.04
JFE	100	37.02 ± 6.51	36.98 ± 5.25	−0.04
JFE	300	36.94 ± 4.00	36.91 ± 4.71	−0.03
ART	4	37.24 ± 2.14	37.21 ± 5.36	−0.03

Values presented as the mean ± SEM, $n = 5$; NC: negative control; ART: artesunate; Δ temperature: change in rectal temperature.

malaria infection than in prophylaxis. Deharo et al. classified *in vivo* antiplasmodial activity of extracts as being very good, good, or moderate if an extract caused parasitaemia suppression of greater than 50% at a dose of 100, 250, and 500 mg·kg^{-1}·day^{-1}, respectively [32]. Based on this criterion, *J. flava* leaf extract can be said to have a very good antiplasmodial activity. Moreover, the extract significantly extended the survival period of mice affirming the overall reduction of parasitaemia [33]. Treatment with JFE also averted significant weight loss and hypothermia in *Plasmodium*-infected animals. The results obtained implied that *J. flava* leaf extract has the ability to suppress parasitaemia and attenuate the pathological events associated with *P. berghei* malaria infection.

Lignans are among plant compounds reported to show significant antimalarial activity against *P. falciparum* and *P. berghei* [34, 35]. From the leaves of *J. flava,* the lignans (+) isolariciresinol (**1**), helioxanthin (**2**), justicinol (**3**), prostalidin D (**4**), 9′-demethylsecolintetralin (**5**), and dimethylsecoisolariciresinol (**6**) were previously isolated [22, 36]. Pyrroloquinazoline alkaloids, vasicinol (**7**) and vasicinone (**8**), and the pyrrolidine alkaloid, hypercratine (**9**), have also been reported from *J. flava* leaves (Figure 7) [22]. In a previous study, vasicinone and vasicine, from *J. adhatoda*, elicited excellent antiplasmodial activity against *P. falciparum* [18]. These phytoconstituents may contribute to the observed antimalarial activity of *J. flava* in this study.

The anti-inflammatory activity of *J. flava* leaves was evaluated by the carrageenan-induced footpad oedema assay, which is a suitable experimental model for inflammation using chicks or rodents [37]. The inflammatory response

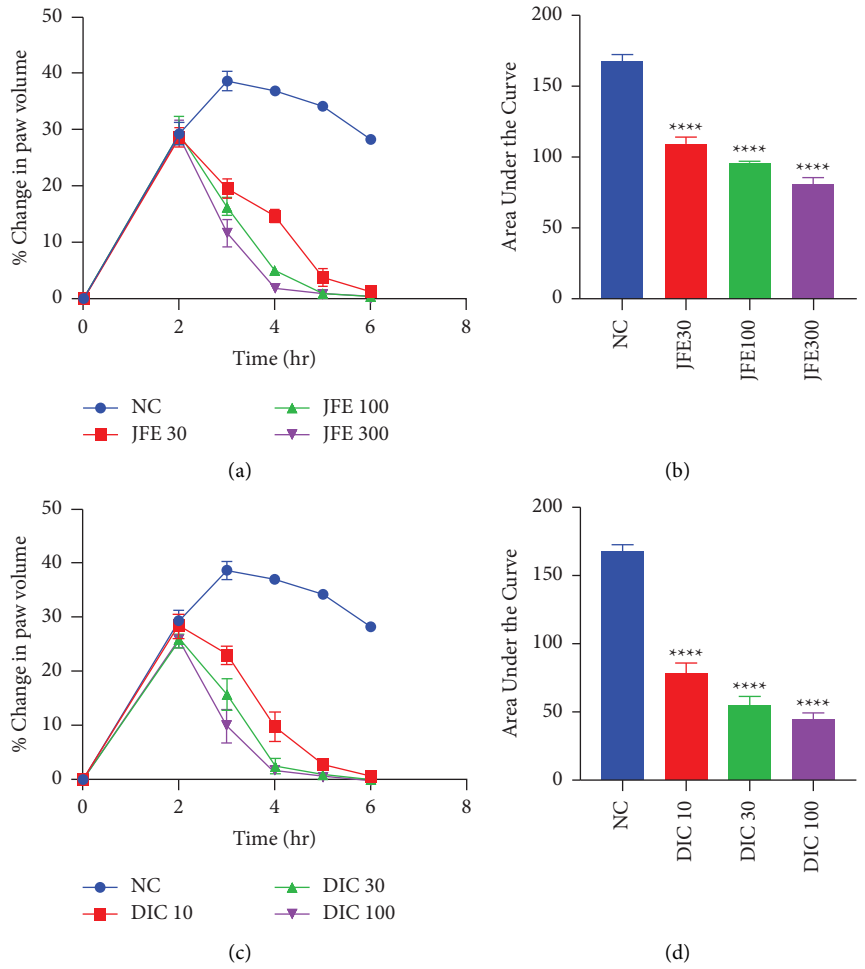

Figure 6: Effect of *J. flava* leaf extract (JFE) and diclofenac sodium (DIC) on the time course curve (a, c) and the total oedema response (AUC) (b, d) in carrageenan-induced oedema in chicks. Data are expressed as the mean ± SEM ($n = 5$). ***** $p < 0.0001$ compared to negative control (saline) (one-way ANOVA followed by Dunnett's post hoc test).

Table 8: Percentage inhibition of oedema in the carrageenan-induced paw oedema assay.

Test sample	Dose ($mg \cdot kg^{-1}$)	% inhibition of oedema
NC	10 $mL \cdot kg^{-1}$	19.46 ± 10.15
JFE	30	38.80 ± 6.51
JFE	100	39.46 ± 5.37
JFE	300	54.00 ± 5.20
DIC	10	53.50 ± 7.80
DIC	30	67.52 ± 6.83
DIC	100	73.88 ± 5.33

Values presented as the mean ± SEM, $n = 5$; NC: negative control; JFE: *J. flava* leaf extract; DIC: diclofenac sodium.

elicited by carrageen follows a biphasic phenomenon: first, the production of serotonin, histamine, and bradykinins within the first 2 hours and a latter phase (3–6 h) depicted by the production of prostaglandins and activation of cyclooxygenase-2 (COX-2) [38]. From the experiment, JFE reduced chick foot pad oedema from the 3rd to the 5th hour of the experiment, implying that the extract may be active in the 2nd phase of inflammation. In a previous study, extracts from *J. flava* leaves inhibited 5-lipooxygenase (5-LOX), the enzyme responsible for the production of leukotrienes from arachidonic acid [24]. Lignans, coumarins, flavonoids, phytosterols, glycosides, and triterpenoids previously isolated from several *Justicia* species have shown promising anti-inflammatory activity in both *in vitro* and *in vivo* models [7, 39–42]. Some mechanisms of action suggested for the anti-inflammatory effect of *Justicia* species include the downregulation of COX-2 and intracellular nitric oxide (iNOS) through the suppression of the NF-κB signalling

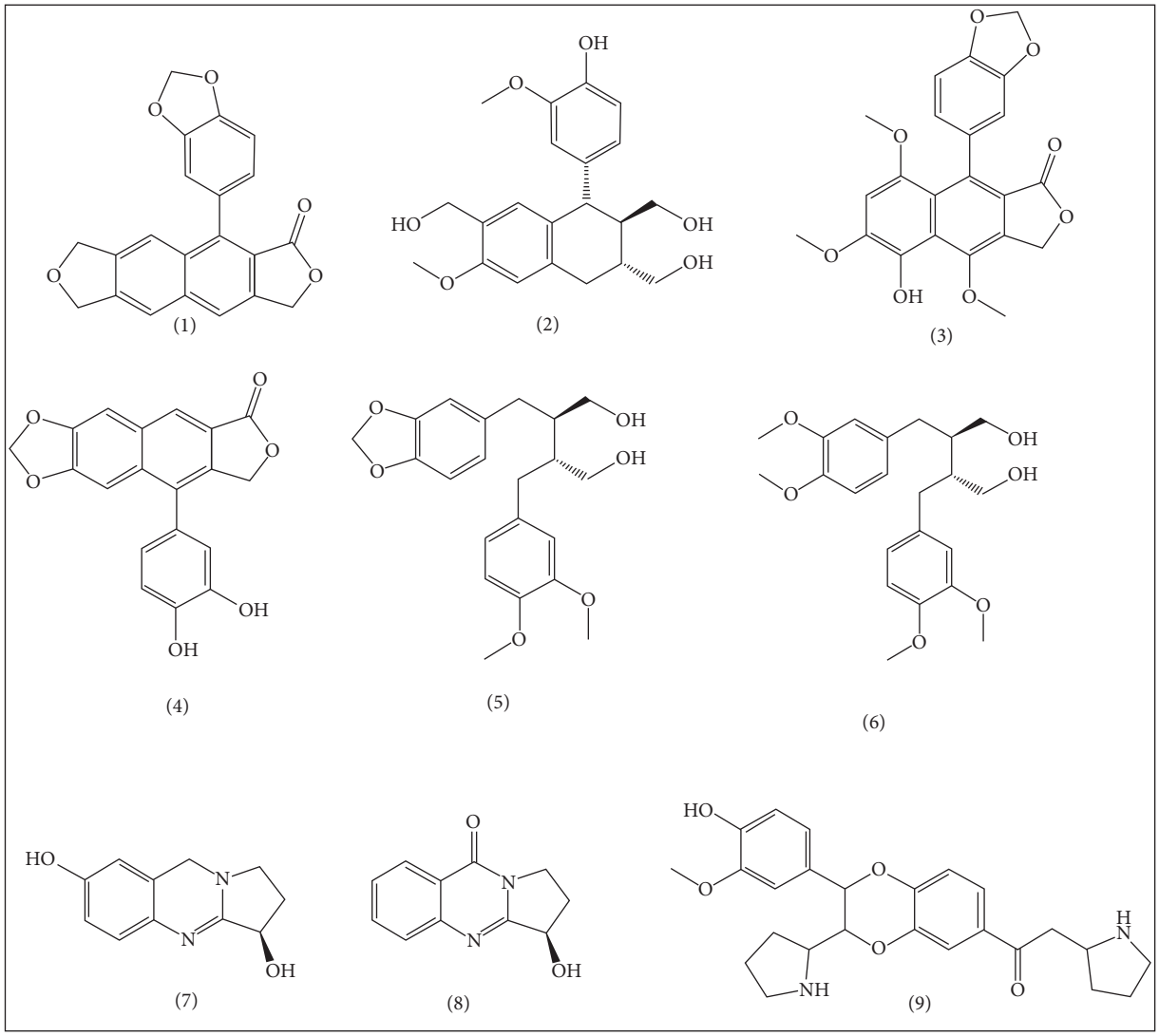

Figure 7: Previously reported phytochemicals from *J. flava* ((+) isolariciresinol (**1**), helioxanthin (**2**), justicinol (**3**), prostalidin D (**4**), 9, 9′-demethylsecolintetralin (**5**), and dimethylsecoisolariciresinol (**6**), vasicinol (**7**), and vasicinone (**8**), and hypercratine (**9**)).

pathway [43], inhibition of the synthesis, or action of tumor necrosis factor-alpha (TNF-α) and cytokine interleukin-1β (IL-β) [44, 45].

From the pharmacognostic investigation of *J. flava*, diacytic stomata, wavy-walled epidermal cells, cylindrical cystoliths, prismatic calcium oxalate crystals, conjoint collateral open vascular bundles, and abundant clothing trichomes were consistent generic features previously observed in most *Justicia* species [46–48]. Only pitted vessels were observed in *J. flava* leaf powder distinguishing this species feature from *J. secunda* [47], *J. adhatoda* [49], and *J. picta* [50] which were reported to have spiral xylem vessels.

5. Conclusion

The antiplasmodial and anti-inflammatory effects of the leaves of *Justicia flava* were verified in this study. The reduction in parasitaemia coupled with the remarkable weight recovery and prolongation of mice survival period approves the folkloric use of *J. flava* for the treatment of malaria. Furthermore, the attenuation of inflammatory response recorded as a decrease in chick foot pad oedema justifies the use of the plant in inflammatory conditions. This finding also points to *J. flava* as a promising source of bioactive antimalarial and anti-inflammatory constituents. The diagnostic pharmacognostic characteristics obtained for the leaves of *J. flava* are important for the correct identification of the plant. This study highlights the significance of bridging traditional knowledge with modern scientific exploration for the improvement of health and well-being.

Data Availability

The raw data/results from experiments used to arrive at the findings of this study are available from the corresponding author upon request. Previous reports that were used to support this study are cited at relevant places within the text as references.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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