The Journal of Phytopharmacology 2023; 12(6):358-365
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(6): 358-365
November- December
Received: 14-10-2023
Accepted: 15-12-2023

doi: 10.31254/phyto.2023.12602

Moussa Ouedraogo
1. Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso
2. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso

Wendwaoga Arsène Nikiema
1. Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso
2. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso

Bonsdawinde Pagbelguem
1. Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso
2. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso

T. Edwige Delma
Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso

Dorcas F. Olusunle
Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso

Apoline Sondo
1. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso
2. Département de Médecine et Spécialités, Service des maladies infectieuses, Centre Hospitalier Universitaire Yalgado Ouedraogo, Ouagadougou, Burkina Faso

R. Armel Flavien Kabore
Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso

Eleonore Kafando
1. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso
2. Laboratoire d'hématologie, Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou, Burkina Faso

Correspondence:
Moussa Ouedraogo
1. Laboratoire de Développement du Médicament, Université Joseph KI Zerbo, Ouagadougou, Burkina Faso
2. Unité de Formation et de Recherche, Sciences de la santé Université Joseph KI-Zerbo, Ouagadougou, Burkina Faso
Email: ouemoussa10@gmail.com

Effects of leaf extracts of *Carica papaya* L. (Caricaceae) and *Vernonia colorata* (Willd.) Drake (Asteraceae) on induced thrombocytopenia and increased vascular permeability: an approach to symptomatic treatment of dengue

Moussa Ouedraogo, Wendwaoga Arsène Nikiema, Bonsdawinde Pagbelguem, T. Edwige Delma; Dorcas F Olusunle, Apoline Sondo, R. Armel Flavien Kabore, Eleonore Kafando

ABSTRACT

Background: Dengue fever is a re-emerging threat that can lead to thrombocytopenia (low platelet count) and severe plasma leakage, sometimes fatal. Supportive care is needed in severe cases, as no specific treatment is yet available. In Burkina Faso, the population commonly uses *Carica papaya* and *Vernonia colorata*. This study aimed to compare the effectiveness of *Vernonia colorata* to *Carica papaya* in reducing the major symptoms of Dengue fever. **Methods**: Lyophilized aqueous ethanolic macerations of fresh leaves of the two plants were prepared, and flavonoid contents were visualized by a fingerprint approach. Platelet and lymphocyte count and the amount of dye leaked from the vascular duct were monitored in pharmacologically induced mouse models. The plant extracts were tested at 100 mg/kg bw on carrageenan (100 mg/kg bw) depressed platelet count on day 2 and at 30 and 100 mg/kg bw on acetic acid-induced dye leakage from the vascular duct. **Results**: The plant extracts at 100 mg/kg bw significantly prevented thrombocytopenia ($p<0.05$) with an increased platelet count on day 2. The acetic acid-increased vascular permeability was inhibited by over 85% ($p<0.001$) in animals treated with 30 and 100 mg/kg bw of each lyophilized plant extract. The effect of *Vernonia colorata* and *Carica papaya* leaf extracts did not differ statistically on thrombocyte count or in preventing increased vascular permeability. A phytochemical fingerprint allowed the characterization of flavonoids in the two plant extracts. **Conclusion**: The leaf extract of *Vernonia colorata* can prevent provoked thrombocytopenia and increased vascular permeability, similar to *Carica papaya*. Further phytochemical content-based molecular mechanisms are expected.

Keywords: *Carica papaya*, Dengue, Thrombocytopenia, Vascular permeability, *Vernonia colorata*.

INTRODUCTION

Dengue fever is a reemergent threat in tropical and subtropical countries. According to the World Health Organization, the number of cases is growing, and approximately 3.9 billion persons in 129 countries are at risk [1]. The number of dengue cases has increased eight times over the past decades, from 505,430 cases in 2000 to 5.2 million cases in 2019 [1]. Approximately 75% of dengue-infected persons are asymptomatic, and 5% evolve to severe dengue. The mortality rate of severe dengue can reach 10% if untreated and 0.1% appropriately if treated [2].

Physiologically, dengue fever is an acute arbovirus infectious disease transmitted through the bite of infected female mosquitoes of the genus *Aedes*. The main vectors are *Aedes aegypti* and *Aedes albopictus*, the daytime-biting mosquito species [3].

Severe forms occur as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue fever is manifested in its severe form by thrombocytopenia and severe plasma leakage, which account for the progression to shock [4]. The dengue fever treatment is symptomatic: rest, patient hydration, medical advice, fever reducers, and painkillers. Severe cases require emergency treatment in a hospital.

However, the first dengue vaccine (CYD-TDV), licensed in December 2015, is targeted at persons living in endemic areas, aged 9 to 45 years and yet not accessible to developing countries' populations [5]. Faced with geographical and financial accessibility difficulties, the majority of populations worldwide have resorted to herbal medicines [6]. Other reasons are cultural habits. Thus, for managing dengue fever and other infectious diseases, populations of Burkina Faso use extemporaneous water maceration of fresh leaves from each or both plants, *Carica papaya* L. (Caricaceae) and *Vernonia colorata* (Willd.) Drake (Asteraceae).

Previous studies reported antioxidant, anti-inflammatory [7,8], anthelmintic [9], larvicidal [10], hepatoprotective [11], nephroprotective [12], hypoglycemic [13] and immunomodulatory [14] effects of *Carica papaya*. *Vernonia colorata* has been reported to have antibacterial [15], anthelmintic [16], hepatoprotective [17], and anti-plasmodial [18] properties.

Recently, in a case report, Carica papaya leaves were successfully used to treat dengue with hemorrhagic manifestations [19]. In a clinical trial, patients treated with *Carica papaya* leaf maceration presented a reduced duration of hospital stay and a significant increase in platelet counts after five consecutive days of treatment [20,21]. Additionally, in laboratory animals, *Carica papaya* leaf extract treatment provoked a significant increase in platelet counts [22]. Other plant species of the genus Vernonia were reported to have beneficial effects in managing dengue infection. The crude extract of *Vernonia anthelmintica* L. wild seeds showed a larvicidal effect on the larvae of *A. aegypti*, one of the Dengue vectors [23]. The methanolic extract of the leaves of *Vernonia lasiopus* increased red blood cells, leukocytes, and platelet counts [24]. Moreover, the ethanolic extract from the leaves of *Vernonia cinerea* showed an inhibitory effect on the proteases of the dengue virus [25].

To our knowledge, no previous study has reported the effect of *V. colorata* leaves in the treatment of dengue. This study aims to verify the ability of *Vernonia colorata* leaf extract, similar to the leaves of *Carica papaya*, to reverse the main symptoms of severe dengue fever, such as thrombocytopenia and increased vascular permeability, induced in animal models.

MATERIALS AND METHODS

Reagents

κ-carrageenan (Cat#22048), indomethacin (Cat#1341001), and Evans blue (Cat#E2129) were purchased from Sigma (RRID:SCR_008988, St Louis MO, USA).

Acetic acid glacial (Cat#131008) for analysis was purchased from PanReac (RRID:SCR_005814) Barcelona, SPAIN.

Quercetin (Cat#Q4951) HPLC grade and tannic acid (#16201) were procured from Sigma (RRID:SCR_008988, St Louis MO, USA).

Ethyl acetate (Cat#23882.330), absolute ethanol (Cat#20821.330), and methanol (Cat#20847.307) were procured from VWR® Chemicals, France. Formic acid (Cat#1.00264.0100) was purchased from VWR® Chemicals, France. 2-Aminoethyldiphenyl borate (Cat# 841636) was purchased from Merck Germany. Polyethylene glycol 4000 (Cat#8.17006) EMPROVE® ESSENTIAL, Ph. Eur. were purchased from Merck, Germany. Distilled water was produced in our laboratory.

Plant sample collection and identification

Fresh mature leaves of *Carica papaya* and *Vernonia colorata* were collected 15 km east of Ouagadougou, the capital of Burkina Faso, in May 2017.

Animals

The different in vivo tests used Swiss albino mice weighing 30-35 g. Female mice were used due to their better sensitivity [26, 27].

The animals were obtained from the International Centre for Research and Development of Livestock in Subhumid Zone (CIRDES) of Bobo-Dioulasso, Burkina Faso. All mice were housed in animal cages at a constant temperature (24°C±2°C) on a 12 h light/dark cycle with food and water available.

Preparation of plant extracts

The popular preparation uses crushed leaves macerated in water for 2 hours in Burkina Faso. The extract is filtered and directly administered to the patient. Based on the population use, the extraction process was improved by increasing the maceration duration to 24 h and using two types of solvents, distilled water and 80% ethanol (v/v). The continuous stirring of the maceration replaced the manual process.

Briefly, 600 g of fresh leaves of each plant was washed thoroughly with tap water, cut into small pieces using scissors and then crushed in a mortar using a pestle. The crushed leaves of each plant were divided into three parts of 200 grams and allowed to macerate in 1000 mL of 80% ethanol (v/v). The maceration lasted 24 hours at 25°C under continuous stirring.

The three extract solution samples were first filtered on tissue gauze and hydrophilic cotton. The obtained filtrates were centrifuged at 1000 × g for 10 min to precipitate insoluble particles. The supernatants collected were concentrated with a rotary vapor system (BUCHI R-114 Labortechnik AG) at 45°C under partial vacuum and then lyophilized using a laboratory freeze-dryer, Alpha 1-4 LSC basic CHRIST.

The extract preparation was repeated three times. The lyophilized extracts from the two plants were used for toxicological and pharmacological tests. The average yield of the three sample extracts was 5,09± 0,11 and 5,35± 0,17 for *Carica papaya* and *Vernonia colorata, respectively.* We prepared aqueous extract for each plant in the same process (results not presented here).

Phytochemical characterization by thin layer chromatography

For standardization purposes, lyophilized *Carica papaya* and *Vernonia colorata* plant extracts were analyzed using chemical markers in the fingerprint thin layer chromatography (TLC) profile approach.

Briefly, 10 mg of a lyophilized extract of each plant was dissolved in 1 ml of water. The chlorophyll was removed with activated charcoal. Quercetin and rutin were used as references. A 0.5 µg/ml methanolic solution of quercetin and rutin was prepared. Five (05) microliters of sample was deposited on aluminum coated with silica gel 60 F_{254} TLC plates (Aluminum TLC plates, Silica gel 60 F254, Cat#1.05554, Merck).

The plate was air-dried and then inserted into a chamber that was presaturated with an optimized mobile phase, a mixed solvent: ethyl acetate/formic acid/acetic acid/water 100/11/11/20 (v/v/v/v). The plate was eluted at room temperature (25°C). After migration, the plate was removed, air-dried, and heated at 105°C for 2 min before being sprayed with Neu's reagent consisting of a mixture of a 1% methanolic solution of 2-aminoethyl diphenyl borate acid and a 5% ethanolic solution of PEG-4000 in a proportion of 10/8 (v/v).

The plate was read using a 366 nm UV lamp (VL-6. LC Vilber RRID:SCR_023580).

Oral acute toxicity assessment

The acute oral toxicity of the two lyophilized extracts was determined using the limit test of OECD guidelines (OECD, 2002a).

Three groups of three (3) female Swiss albino mice consisted of one control and two tests. The acute toxicity through lethal dose (LD_{50}) was approached by a single oral gavage of 2000 mg/kg bw of each lyophilized plant extract to the test groups. The control group received distilled water. All groups had access to water and food after administering the treatments 2 hours later. Mice were observed from 2-72 h and two weeks for morbidity or mortality, and changes in behavior were recorded compared to the control group treated with water. The experiment was repeated twice.

Mouse model of carrageenan-induced thrombocytopenia

The thrombocytopenia induced by carrageenan previously described in rats [28] was adapted in mice in our laboratory for availability reasons. Eighteen [18] mice were randomly divided into three [3] groups of six mice: one control and two test groups. The test groups received one injection of 100 or 150 mg/kg bw carrageenan by the intraperitoneal route. The control group received sodium chloride solution at 0.9%.

Animals were anesthetized with thiopental (NEON laboratories limited) (50 mg/kg.bw by intraperitoneal route) to allow blood sample collection by a retro-orbicular puncture. Samples were collected at times T0 before and 2 hours, day 1, day 2, day 5, and day seven after the carrageenan challenge.

The blood samples were carried to the biomedical analysis laboratories of Charles de Gaulle Pediatric University Hospital for hematological analysis. An automat Micros ABX 60® allowed platelet and white cell counts.

Plant extract effects on carrageenan-induced thrombocytopenia.

A mouse model of carrageenan-induced thrombocytopenia reproduced in our laboratory was used.

The ability of plant extracts to prevent the decrease in thrombocytes was evaluated on day two, as the depression thrombocyte count was maximal. Thus, six (06) groups of six mice were randomly constituted: two controls (negative and positive), two plant extract control groups and two test groups. The two test groups and the positive control group received one injection of 100 mg/kg bw carrageenan by the intraperitoneal route. The control groups (negative, positive, plant extract controls) received a 0.9% NaCl solution. Sixty (60) min before the plant extracts, the control and test groups were treated orally with 100 mg/kg bw of a lyophilized extract of *Carica papaya* or *Vernonia colorata*. The treatment of groups with plant extracts was given daily for five days. The negative and positive control groups received distilled water. Blood samples were collected on days 1, 2, and 5 to allow platelet and white cell counts using the abovementioned method.

Plant extract effect on acetic acid-induced vascular permeability in mice

An in vivo vascular permeability test described previously by Whittle BA [29] was used.

Six (6) groups of six (6) female mice previously fasted for 16 hours were established. Four test groups orally received 30 mg/kg bw and 100 mg/kg bw of each lyophilized extract of *Carica papaya* or *Vernonia colarata*.

A reference group was given 100 mg/kg bw indomethacin. The control group received a 0.9% NaCl solution. All treatments were given by the oral route. One (1) hour after administration, each mouse received 0.1 ml/10 g of body weight of 0.5% Evans blue dye in 0.9% NaCl solution by the intravenous route. Then, 15 minutes after administering the vital dye, all animals received acetic acid 0.6% in water at a dose of 0.1 ml/10 g body weight by the intraperitoneal route. Thirty (30) minutes later, the animals were euthanized by vapors generated by cotton soaked with petroleum ether. The skin of the abdomen of each mouse was carefully incised, and the abdominal cavity was rinsed (3x2 ml) with a 0.9% NaCl solution to gather the leaked dye. The rinsed solution was collected and centrifuged at 3500 rpm for 10 minutes. Then, the optical density of the supernatant was read at 600 nm using a microplate spectrophotometer (BioTek Epoch Microplate Spectrophotometer RRID:SCR_019741). We determined the amount of leaked Evan's blue dye using a calibration curve ranging from 0.5 to 12 µg/l. The results are expressed as the mean ± sem of the amount of Evan's blue dye leaked in the animal abdominal cavity (µg/g of the mouse) or as the percentage of inhibition of vascular permeability according to the formula:

Inhibition (%) = (CT-Ct)/CT x 100

CT = concentration of Evan's blue dye in the control group, and Ct = concentration of Evan's blue dye in the test group.

Statistical analysis

Statistical analysis was executed by one-way ANOVA (when comparing doses) or two-way (for treatment and time comparison) test with Bonferroni posttest using the software GraphPad Prism version 5.03 (RRID:SCR_002798, GraphPad Software Inc., San Diego, California) with a *p* value <0,05.

RESULTS

Qualitative phytochemical screening of compounds

The TLC profile showed yellow spots under ultraviolet 366 nm in the aqueous ethanolic extracts of *Carica papaya* and *Vernonia colorata*. Additionally, green and fluorescent spots were observed with *Carica papaya* leaf extract (Figure 1). The two plant extracts shared a spot with a retention factor of 0.87 near the Rf of quercetin (R_f 0.90). Rutin (R_f 0,43) was retrieved in *Carica papaya* but not in *Vernonia colorata*.

Acute toxicity by the oral route

The lyophilized extract of plants at 2000 mg/kg bw by the oral route did not provoke animal death or adverse toxic manifestations. All animals survived after 14 days of observation. The LD_{50} of the extract of leaf *Carica papaya* L. (Caricacea) or *Vernonia colorata* (Willd.) Drake (Asteraceae) was estimated as more than 5000 mg/kg.bw.

Carrageenan induced-thrombocytopenia

Carrageenan given by the intraperitoneal route at 100 or 150 mg/kg bw to mice provoked a decrease in platelet counts on days 1 and 2 (Figure 2). The platelet counts recovered to typical values on day 5. The reduction in the thrombocyte count was significant ($p<0.05$) on day two after the carrageenan challenge.

The tested doses of carrageenan also significantly modified the count of white blood cells, such as lymphocytes, monocytes, and granulocytes (Table 1). The monocyte and granulocyte count significantly increased, while the lymphocyte count decreased. The decrease in the thrombocyte count did not differ between the tested doses. The dose of 100 mg/kg bw of carrageenan was retained for our model of thrombocytopenia to evaluate the effects of the *C. papaya* and *V. colorata* leaf extracts.

Table 1: Carrageenan administered intraperitoneally changed the white cell counts in mice

Treatment	Doses (mg/kg.bw)	Counts (x10^3/mm^3)		
		Lymphocytes	Monocytes	Granulocytes
Control	Distilled water	4.47 ±0.4	0.83 ±0.13	0.95±0.12
Carrageenan	100	3.02±0.23**	1.36±0.17*	3.7±0.49*
	150	3.05±0.6**	3.1±0.23**	2.2±0.32*

*p<0.05, **p<0,01 Carrageenan versus Control

Plant extract effect on carrageenan-induced changes in leukocyte count and thrombocytopenia at 48 h

The platelet counts in groups of plant extract controls receiving 100 mg/kg bw of lyophilized leaf extract of *Carica papaya* or *Vernonia colorata* compared to the negative control group treated with distilled water was unaffected. In the groups challenged with carrageenan, treatment with 100 mg/kg bw of lyophilized leaf extract of *Carica papaya* or *Vernonia colorata* prevented the decrease in platelet count provoked by carrageen ($p<0,05$) (Figure 3). The effect of the two plant extracts on induced thrombocytopenia did not differ statistically.

The leukocyte count did not change in mice treated with lyophilized plant extracts. In groups challenged by carrageenan, the depression of lymphocyte count was prevented by *Carica papaya* leaf extract but not by *Vernonia colorata leaf extract.* The two plant extracts contained a carrageenan-induced monocyte increase, and granulocyte counts decreased (Table 2).

Table 2: Effects of *C. papaya* and *V. colorata* lyophilised extracts on white cell counts in the presence or absence of carrageenan shot on day two.

Treatment	Doses (mg/kg.bw)	Counts (x10^3/mm^3)		
		Lymphocytes	Monocytes	Granulocytes
Control	Distilled water	4.47±0.40	0.83±0.13	0.95± 0.12
Carr	100	3.02±0.23**	1.30±0.10*	3.70±0.49**
Carr + Vc	100+100	1.93±0.80$^{\$}$	0.90±0.80	1.46±1.80
Carr + Cp	100+100	3.80±2.80	1.08±0.66	1.95±1.40
Vc	100	4.13±1.10	0.53±0.20	1.13±0.50
Cp	100	4.46±1.40	1.03±0.47	1.38±0.60

Carrageenan (Carr), *Vernonia colorata* (Vc), *Carica papaya* (Cp), *p<0.05, **p<0,01 Carrageenan versus Control; $^{\$}$ p<0.05 *Carr + Vc* versus Control

Effect of plant extracts on acetic acid-induced vascular permeability

The acetic acid injected intraperitoneally into the animals provoked the leakage of Evan's blue dye, previously administered intravenously to the abdominal cavity. Compared to the control group, each lyophilized plant extract at the tested doses of 30 and 100 mg/kg bw inhibited more than 85% of the plasma leakage provoked by acetic acid injection ($p<0,0001$). The effects of the tested doses of 30 and 100 mg/kg bw of the *Carica papaya* leaf extract but not of the *Vernonia colorata* leaf extract were not different between doses and plant extract. The importance of the permeability inhibition was similar, as did the reference. (Figure 4).

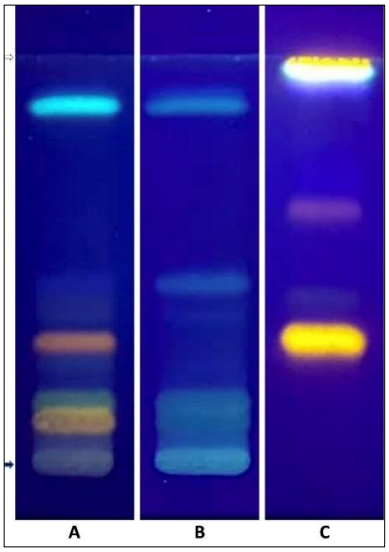

Figure 1: TLC fingerprint of lyophilized extract of leaves of *Carica papaya* and *Vernonia colorata*. Legend: Depot line (filled arrow) Front of solvent (opened arrow) samples were *Carica papaya* (A), *Vernonia colorata* (B), mixed references, quercetin and rutin (C), which were revealed by NEU's reagent using 366 nm UV light.

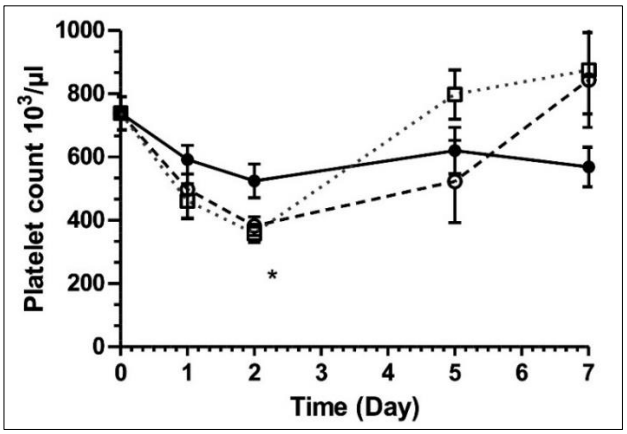

Figure 2: Carrageenan-induced decrease in platelet count in mice as a thrombocytopenia model The control (continuous line-filled circle symbol); Carrageenan 100 mg/kg.bw (dashed line opened circle); Carrageenan 150 mg/kg.bw (dotted line opened square). *P<0.05 Ctrl versus 100 mg/kg bw Carr or 150 mg/kg bw Carr on day 2

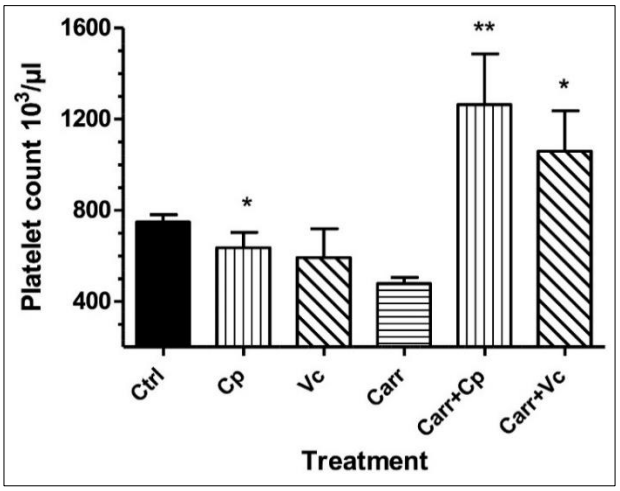

Figure 3: Effect of 100 mg/kg bw of lyophilized aqueous ethanolic leaf extract of *C. papaya* and *V. colorata* on platelet count on Day 2 with or without carrageenan challenge. Carr: carrageenan; *Cp: Carica papaya*; *Vc*: *Vernonia colorata*. *p<0.05, 100 mg/kg bw Carr vs 100 mg/kg bw Carr+ Vc; **p<0.001, 100 mg/kg bw Carr vs 100 mg/kg bw Carr+Cp; $p<0.05, Ctrl vs 100 mg/kg bw Carr

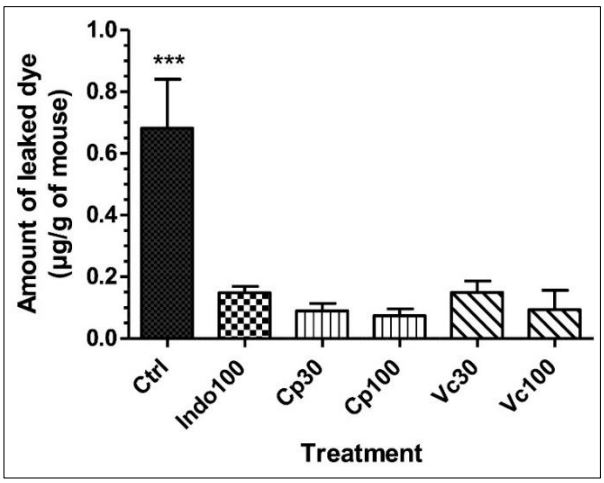

Figure 4: Effect of lyophilized aqueous ethanolic extracts of *C. papaya* and *V. colorata* on acetic acid-induced vascular leakage of Evans blue dye. Control (Ctrl); indomethacin (Indo); *V. colorata* (Vc); *C. papaya* (Cp); ***p<0.0001 Ctrl in comparison to all conditions of treatment.

DISCUSSION

This study evaluated the safe use and effect of lyophilized aqueous ethanolic extract of *Vernonia colorata* compared to *Carica papaya* extract on thrombocytopenia and plasma leakage in a mouse model.

The lyophilized aqueous ethanolic extract of *Vernonia colorata* or *Carica papaya* is not toxic by the oral route. In Burkina, populations use fresh leaf aqueous extract of *Vernonia colorata, Carica papaya,* or a combination to treat dengue fever. We compared the effect of the extract of *Vernonia colorata* to *Carica papaya* in mouse models reproducing severe dengue conditions with pharmacological tools. The extraction solvent was improved using ethanol/water (80:20) (v/v). The water extract in our experimental conditions yielded weaker efficacy (results not shown). We found that *Vernonia colorata* aqueous ethanolic extract contained carrageenan-induced thrombocytopenia and prevented the acetic acid-provoked increase in vascular permeability. These effects did not differ from those of the aqueous ethanolic extract *of Carica papaya*.

Plant extract toxicity assessment

The lyophilized aqueous ethanolic leaf extract of *C. papaya* L. (Caricacea) or *V. colorata* (willd.) drake (Asteraceae) by the oral route presented an estimated LD_{50} beyond 5000 mg/kg bw in our experimental conditions using OECD guidelines [30]. Other authors reported that the oral administration of increasing doses of 5, 50, 300 and 2000 mg/kg bw of aqueous leaf extract of *C. papaya to* Sprague Dawley rats showed no sign of toxicity and no deaths [31]. The DL_{50} was estimated to be higher than 5000 mg/kg bw in Swiss albino mice receiving 70% methanolic crude extract of *V. colorata by the oral route* [32]. According to the Global Classification and Harmonization System (GHS) [30], lyophilized aqueous ethanolic extracts of *C. papaya* or *V. colorata* are not toxic.

The LD_{50} of the aqueous ethanolic leaf extract of *C. papaya* or *V. colorata* is beyond 5000 mg/kg bw by the oral route in mice allowed to test pharmacological doses up to 1/100 of the DL_{50}. In our pharmacological tests, we used a maximal dose of 100 mg/kg bw for the two extracts.

Plant extracts reversed carrageenan-induced thrombocytopenia

Thrombocytopenia is a condition defined by a significant drop in the number of blood platelets due to a deficit of production, increased consumption or sequestration of platelets [33]. The model of carrageenan-induced thrombocytopenia has been stated as platelet sequestration or consummation in disseminated intravascular coagulation [34]. The studied plant extracts challenged carrageenan-induced thrombocytopenia. One could not exclude a triggering action of plant extracts on megakaryocytopoiesis in carrageenan-induced stress.

Thrombocytopenia is present in many diseases or situations. Infectious disease-caused thrombocytopenia includes chikungunya, dengue, Ebola, hemorrhagic fever, and leptospirosis. Additionally, a vital drop in platelet counts can occur in stress, sepsis, and idiopathic thrombocytopenic purpura [35–37]. In its severe form, dengue fever presents a biological picture associating significant thrombocytopenia with plasma leakage [38]. The specific mechanism by which dengue virus causes thrombocytopenia has yet to be well understood. However, different activities mediated by the virus in the host system suggested either inhibition of megakaryocyte maturation by the

dengue virus or increased platelet aggregation [39, 40]. Dengue virus may also cause platelet activation, mitochondrial dysfunction and apoptosis [41–43].

Our results showed that *Vernonia colorata* (Willd.) Drake leaf extract could prevent carrageenan-induced thrombocytopenia, as did *Carica papaya* L. leaf extract. The plant extract-based mechanism seems inversely related to platelet peripheral fate with possible action on direct megakaryocytopoiesis, as this process lasts 4 to 6 days. Such a mechanism evokes cytokine-based central action [44]. A previous study showed that extracts from *Carica papaya* stimulate the production of the cytokines IL-3 and IL-6 and stem cell factor, which induces thrombocytosis [45]. Carpaine and alkaloidal extracts from *Carica papaya* were reported to prevent thrombocytopenia [46].

Plant extracts on increased vascular permeability

Plasma leakage is a precursor to life-threatening complications of dengue fever. It gives rise to shock and organ failure. Hence, plasma leakage prevention is critical in managing dengue fever. The mechanisms that induce that alteration are diverse. Endothelial cells, central elements in the metabolism and physiology of tissues and organs, are first affected in viral infections by cell death after membrane dysfunction. Direct invasion of myeloid cells and overactivation of T cells by the dengue virus induce the release of large amounts of cytokines, qualified as a cytokine storm [47]. These cytokines (IL6, TNFα) and other mediators, such as histamine and tryptase protein, disrupt cell junctions [48-50].

The lyophilized aqueous ethanolic extract of *Carica papaya* or *Vernonia colorata* significantly reduced plasma leakage in the animal model. At the tested doses of 30 and 100 mg/kg bw, the effects of the two plant extracts did not differ statistically. The effective dose could be less than or equal to 30 mg/kg bw. The plant extract flavonoid content could contribute to preventing the effect of plasma leakage. Conventional medicines made of plant flavonoids are used to improve vein tonus.

Our results improve knowledge of medicinal plants for managing severe dengue fever symptoms, which are reported to affect more than 500,000 people annually, including many children, with a lethality of 2.5% [51]. More is already known about *Carica papaya* but not about *Vernonia colorata*. Further investigations are needed to highlight the role of the chemical compounds of *V. colorata* extract in thrombocytosis and preventing an increase in vascular permeability. Additionally, one could not discard a synergistic action in combining the two plant extracts for managing Dengue fever, as do some populations in Burkina Faso.

Phytochemical fingerprint analysis

Comparing the TLC profile of the two plant extracts, *Vernonia colorata* extract contains a few flavonoid spots. Active flavonoids such as quercetin, quercitrin kaempferol, alkaloids (carpaine), coumarins, and enzymes (papain) have been isolated from *Carica papaya* [52,53]. The role of these chemical compounds is critical in the Dengue fever management properties of leaves of *Carica papaya* due to the direct inhibition of such compounds on virus proteases, thrombocytosis effects, or interference with the blood-clotting system [54]. Reported phytochemical group contents of ethanolic extracts of leaves of *Vernonia colorata* include tannins, glycosides, saponins, alkaloids, flavonoids, sterols (terpenoid nucleus), and cardiac glycosides [55]. This chemical group content is close to that of the chemical groups retrieved from the leaves of *Carica papaya*: alkaloids, terpenoids, phenols, tannins, flavonoids, saponins, and glycosides [56,57]. Our results show that *Vernonia colorata* leaf aqueous ethanolic extract has a similar effect to *Carica papaya* leaf extract on provoked thrombocytopenia or increased vascular permeability.

Declarations

Ethics approval and consent to participate

Not applicable.

All animal welfare and experiments were strictly performed in accordance with the Guide for the Procedures approved by the Animal Ethics Committee, Joseph Ki Zerbo University of OUAGADOUGOU (Approval No. CE-UJKZ/2017-03). All experiments were conducted in accordance with international standards of animal welfare as recommended by the European Union on Animal Care (EEC 86/609, UE 2010/63).

Plant collection and Authentication

Identification and authentication of the plants' specimens was done by a taxonomist by Prof. A. Ouedraogo of the Laboratory of Plant Biology and Ecology of the University Joseph KI-ZERBO of Ouagadougou. Voucher specimens were deposited under reference numbers 6986/18026 for *Vernonia colorata (*Willd.) Drake. (Asteraceae) and 6987/18027 for *Carica papaya* Linn. (Caricaceae)

Consent for publication

Not applicable.

Data Availability

The data used and analyzed in this study are available from the corresponding author on reasonable request.

Competing interests

The author declares that there is no conflict of interest.

Funding

This work did not receive any fund from third party.

Authors' contribution

OM and NA, both wrote design processed the different experiments, analysed data and wrote the first draft; PB participate in conception, design and data processing; DE and OD wrote sections of the manuscript; SA, KF, and KE conceived biological tests and validated the data. All authors contributed to manuscript revision and read and approved the submitted version.

Acknowledgments

We want to thank the research team of the Drug Development Laboratory of Joseph KI-ZERBO University and the biology team of the Hematology Laboratory of Charles de Gaulle Pediatric University Hospital. Dr Kiran Jadhav, KLE College of Pharmacy, Belgaum, India, is acknowledged for her critical manuscript reading.

CONCLUSION

Animal models of provoked thrombocytopenia and increased vascular permeability allow us to confirm the beneficial effect of the leaves of *Vernonia colorata compared to Carica papaya* in managing the main symptoms of severe dengue fever. More understanding is expected with refined chemical analysis and mechanistic *Vernonia colorata* leaf extract studies.

Conflict of Interest

None declared.

Financial Support

None declared.

ORCID ID

Moussa Ouedraogo: https://orcid.org/0000-0002-3534-8301
Wendwaoga Arsène Nikiema: https://orcid.org/0000-0002-6128-7274
Bonsdawinde Pagbelguem: https://orcid.org/0009-0002-1127-6275
T. Edwige Delma: https://orcid.org/0009-0000-3692-3745
Dorcas F Olusunle: https://orcid.org/0000-0001-8731-7606
Apoline Sondo: https://orcid.org/0000-0002-1136-9917
R. Armel Flavien Kabore: https://orcid.org/0000-0002-5084-2121
Eleonore Kafando: https://orcid.org/0000-0001-9053-437X

REFERENCES

1. WHO Dengue and severe dengue. Geneva 2019.
2. Sahak MN. Dengue fever as an emerging disease in Afghanistan: Epidemiology of the first reported cases. Int J Infect Dis 2020; 99:23–27.
3. Kraemer MU, Sinka ME, Duda KA, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife 2015; 4:e08347.
4. de Azeredo EL, Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators. Mediators Inflamm 2015; 2015:313842.
5. Thomas SJ, Yoon I-K. A review of Dengvaxia®: development to deployment. Hum Vaccines Immunother 2019; 15: 2295–2314.
6. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014; 4: 177.
7. Adedapo. A., Orherhe V. Antinociceptive and anti-inflammatory studies of the aqueous leaf extract of Carica papaya in laboratory animals. Asian J Exp Biol Sci 2013; 4: 89–96.
8. Ranasinghe P, Ranasinghe P, Abeysekera WPKM, et al. In vitro erythrocyte membrane stabilization properties of Carica papaya L. leaf extracts. Pharmacogn Res 2012; 4: 196–202.
9. Ameen SA, Azeez OM, Baba YA, et al. Anthelmintic Potency of *Carica papaya* seeds against Gastro-intestinal Helminths in Red Sokoto goat. Ceylon J Sci 2018; 47: 137.
10. Wahyuni D. New Bioinsecticide Granules Toxin from Ectract of Papaya (Carica Papaya) Seed and Leaf Modified Against Aedes Aegypti Larvae. Procedia Environ Sci 2015; 23: 323–328.
11. Sadeque MZ, Begum ZA. Protective effect of dried fruits of Carica papaya on hepatotoxicity in rat. Bangladesh J Pharmacol 2010; 5: 48–50.
12. Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. Afr Health Sci 2015; 15: 598–605.
13. Solikhah TI, Setiawan B, Ismukada DR. Antidiabetic Activity of Papaya Leaf Extract (Carica Papaya L.) Isolated with Maceration Method in Alloxan- Induces Diabetic Mice. Syst Rev Pharm 2020; 11: 5.
14. Aziz J, Abu Kassim NL, Abu Kasim NH, Haque N, Rahman MT. Carica papaya induces in vitro thrombopoietic cytokines secretion by mesenchymal stem cells and hematopoietic cells. BMC Complement Altern Med 2015; 15: 215.
15. Rabe T, Mullholland D, van Staden J. Isolation and identification of antibacterial compounds from Vernonia colorata leaves. J Ethnopharmacol 2002; 80: 91–94.
16. Morah F, Ogar A, Nathaniel H, et al. Chemical composition, anthelmintic, insecticidal and antimicrobial activities of Vernonia colorata leaf essential oil. 2019; 7: 45–50.
17. Ijeh II, Onyechi O. Biochemical and histopathological changes in liver of albino rats fed diets incorporated with Vernonia amygdalina and Vernonia colorata leaves. Int J Med Med Sci 2010; 2: 285–289.
18. Chukwujekwu JC, Lategan CA, Smith PJ, Van Heerden FR, Van Staden J. Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of Vernonia colorata. South Afr J Bot 2009; 75: 176–179.
19. Ahmad N, Fazal H, Ayaz M, Abbasi BH, Mohammad I, Fazal L. Dengue fever treatment with Carica papaya leaves extracts. Asian Pac J Trop Biomed 2011; 1: 330–333.
20. Gowda AC, Hospital P, Nagar R, et al. A Pilot Study to Evaluate the Effectiveness of Carica Papaya Leaf Extract in Increasing the Platelet Count in Cases of Dengue with Thrombocytopenia. Indian Med Gaz 2015; 8.
21. Kasture PN, Nagabhushan KH, Kumar A. A Multicentric, Double-blind, Placebo-controlled, Randomized, Prospective Study to Evaluate the Efficacy and Safety of Carica papaya Leaf Extract, as Empirical Therapy for Thrombocytopenia associated with Dengue Fever. J Assoc Physicians India 2016; 64: 15–20.
22. Patil S, Shetty S, Bhide R, Narayanan S. Evaluation of Platelet Augmentation Activity of Carica papaya Leaf Aqueous Extract in Rats. J. Pharmacogn. Phytochem. 2013, 5.
23. Hellert A, Sharma G, Kumar K, Agrawal V. Exploration of larvicidal activity of Vernonia anthelmintica (L.) wild seed crude extracts in different solvents against malaria (Anopheles stephensi) and dengue (*Aedes aegypti*) vectors. Malar J 2012; 11: P46, 1475-2875-11-S1-P46.
24. Muriithi N, Maina G, Maina M, et al. Determination of Hematological Effects of Methanolic Leaf Extract of Vernonia lasiopus in Normal Mice. J Blood Lymph 2015; 05.
25. Rothan HA, Zulqarnain M, Ammar YA, Tan EC, Rahman NA, Yusof R. Screening of antiviral activities in medicinal plants extracts against dengue virus using dengue NS2B-NS3 protease assay. Trop Biomed 2014; 31: 286–296.
26. OECD. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. OECD, 2002.
27. Leng X-H, Hong SY, Larrucea S, et al. Platelets of female mice are intrinsically more sensitive to agonists than are platelets of males. Arterioscler Thromb Vasc Biol 2004; 24: 376–381.
28. Davidson RJ, Simpson JG, Whiting PH, Milton JI, Thomson AW. Hematological changes following systemic injection of purified carrageenans (kappa, lambda and iota). Br J Exp Pathol 1981;62:529–539.

29. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. Br J Pharmacol Chemother 1964; 22: 246–253.
30. OECD. Guidance Document on Acute Oral Toxicity Testing. OECD, 2002.
31. Halim SZ, Abdullah N, Afzan A, Abd Rashid B, Jantan I, Ismail Z. Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats. J Med Plants Res 2011; 5: 1867–1872.
32. Idris MH, Mann A, Kabiru AY, Busari MB. In vivo Antiplasmodial Activity and GC-MS Analysis of Vernonia colorata (Willd) Drake Leaf. Eur J Med Plants 2016; 1–11.
33. Weiss DJ, Wardrop KJ, Schalm OW (eds). Preclinical evaluation of compound-related cytopenias. 6th ed. Wiley-Blackwell: Ames, Iowa, 2010.
34. Kuznetsky RD, Trobaugh JF, Adler SS. Effects of carrageenan on the mouse hematopoietic system. Exp Hematol 1980; 8: 465–476.
35. Chow A, Her Z, Ong EKS, et al. Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J Infect Dis 2011; 203: 149–157.
36. Rajendran P, Rengarajan T, Thangavel J, et al. The Vascular Endothelium and Human Diseases. Int J Biol Sci 2013; 9: 1057–1069.
37. Parikh F. Infections and Thrombocytopenia. J Assoc Physicians India 2016; 64: 11–12.
38. Castilho BM, Silva MT, Freitas ARR, Fulone I, Lopes LC. Factors associated with thrombocytopenia in patients with dengue fever: a retrospective cohort study. BMJ Open 2020; 10: e035120.
39. de Araújo JMG, Schatzmayr HG, de Filippis AMB, et al. A retrospective survey of dengue virus infection in fatal cases from an epidemic in Brazil. J Virol Methods 2009; 155: 34–38.
40. Lin G-L, Chang H-H, Lien T-S, et al. Suppressive effect of dengue virus envelope protein domain III on megakaryopoiesis. Virulence 2017; 8: 1719–1731.
41. Hottz ED, Oliveira MF, Nunes PCG, et al. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. J Thromb Hemost JTH 2013; 11: 951–962.
42. Hottz ED, Bozza FA, Bozza PT. Platelets in Immune Response to Virus and Immunopathology of Viral Infections. Front Med 2018; 5: 121.
43. Andrews RK, Arthur JF, Gardiner EE. Neutrophil extracellular traps (NETs) and the role of platelets in infection. Thromb Hemost 2014; 112: 659–665.
44. Guo T, Wang X, Qu Y, Yin Y, Jing T, Zhang Q. Megakaryopoiesis and platelet production: insight into hematopoietic stem cell proliferation and differentiation. Stem Cell Investig 2015; 2: 3.
45. Aziz J, Abu Kassim NL, Abu Kasim NH, Haque N, Rahman MT. Carica papaya induces in vitro thrombopoietic cytokines secretion by mesenchymal stem cells and hematopoietic cells. BMC Complement Altern Med 2015; 15: 215.
46. Zunjar V, Dash RP, Jivrajani M, Trivedi B, Nivsarkar M. Antithrombocytopenic activity of carpaine and alkaloidal extract of Carica papaya Linn. leaves in busulfan induced thrombocytopenic Wistar rats. J Ethnopharmacol 2016; 181: 20–25.
47. Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. Semin Immunopathol 2017; 39: 563–574.
48. Ashina K, Tsubosaka Y, Nakamura T, et al. Histamine Induces Vascular Hyperpermeability by Increasing Blood Flow and Endothelial Barrier Disruption In Vivo. Plos one 2015;16.
49. Rathore APS, Syenina A, Gubler D, John ALS. Dengue virus-elicited tryptase breaks tight junctions to induce endothelial permeability. J Immunol 2016; 196: 217.19-217.19.
50. Rathore APS, Mantri CK, Aman SAB, et al. Dengue virus–elicited tryptase induces endothelial permeability and shock. J Clin Invest 2019; 129: 14.
51. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013; 496: 504–507.
52. Akhila S, vijayalakshmi. phytochemical studies on carica papaya leaf juice. Int J Pharm Sci Res 2015; 6: 880–883.
53. Teng W-C, Chan W, Suwanarusk R, et al. In vitro Antimalarial Evaluations and Cytotoxicity Investigations of Carica papaya Leaves and Carpaine. Nat Prod Commun 2019; 14: 1934578X1901400110.
54. Sarker MdMR, Khan F, Mohamed IN. Dengue Fever: Therapeutic Potential of Carica papaya L. Leaves. Front Pharmacol 2021; 12: 610912.
55. Kwasi Adu J KT Abena Brobbey, Cedric Amengor, Yaw Duah. Resistance modulation studies of vernolide from Vernonia colorata(Drake) on ciprofloxacin, amoxicillin, tetracycline and erythromycin. The Journal of Phytopharmacology 2018; 7.
56. Ikeyi AP, Ogbonna AO, Eze FU. Phytochemical analysis of paw-paw (Carica papaya) leaves. Int J Life Sci Biotechnol Pharma Res 2013; 2: 347–351.
57. Jaji SO, Damazio OA, Aiyelero TS, et al. Phytochemical Contents, Characterization and Elemental Analysis of Pawpaw Leave Extract (Carica papaya). J Chem Soc Niger 2020; 45.

HOW TO CITE THIS ARTICLE

Ouedraogo M, Nikiema WA, Pagbelguem B, Delma TE, Olusunle DF, Sondo A, Kabore RAF, Kafando E. Effects of leaf extracts of *Carica papaya* L. (Caricaceae) and *Vernonia colorata* (Willd.) Drake (Asteraceae) on induced thrombocytopenia and increased vascular permeability: an approach to symptomatic treatment of dengue. J Phytopharmacol 2023; 12(6):358-365. doi: 10.31254/phyto.2023.12602