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

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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-induced 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. Faced with geographical and financial accessibility difficulties, the majority of populations worldwide have resorted to herbal medicines. 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-Aminoethylphenyl 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 CHIRST.

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 F254 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% methanol 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 (LD₅₀) 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 [28] 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 thromocyte 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 colorata*.

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:

\[
\text{Inhibition (\%)} = \frac{(\text{CT} - \text{Ct})}{\text{CT}} \times 100
\]

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 (Rf 0.90). Rutin (Rt 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 LD₅₀ 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg.bw)</th>
<th>Lymphocytes (x10⁶/mm³)</th>
<th>Monocytes (x10⁶/mm³)</th>
<th>Granulocytes (x10⁶/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Distilled water</td>
<td>4.47±0.4</td>
<td>0.83±0.13</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>100</td>
<td>3.02±0.23**</td>
<td>1.36±0.17*</td>
<td>3.7±0.49*</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3.05±0.6**</td>
<td>3.1±0.23**</td>
<td>2.2±0.32*</td>
</tr>
</tbody>
</table>

*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 carrageenan (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).

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<0.001). 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg.bw)</th>
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<tr>
<td>Carr + Vc</td>
<td>100</td>
<td>3.02±0.23**</td>
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<td>3.7±0.49*</td>
</tr>
<tr>
<td>Carr + Cp</td>
<td>100</td>
<td>3.05±0.6**</td>
<td>3.1±0.23**</td>
<td>2.2±0.32*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 Carrageenan versus Control; p<0.05 Carr + Vc versus Control

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.

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.
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. (Caricaceae) 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 consumption in disseminated intravascular coagulation [34]. The studied plant extracts challenged carrageenan-induced thrombocytopenia. One could not exclude a triggering action of plant extracts on megakaryocytopenesis 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 megakaryocytopenia, 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 [42,53]. 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 OUAGADOGOU (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.

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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

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REFERENCES

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.
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