INTRODUCTION

In alternative medicine, plants are a source of bioactive compounds. In fact, 80% of the population in developing countries rely on these bioactive compounds for their healthcare 1. Gastrointestinal diseases also figure prominently among the population's various ailments. In 2019, there were 8 million deaths from digestive diseases, and this figure is not in decline over the last three decades 2. The spasmolytic effect of drugs is commonly used to reduce excessive contractility of the intestine, responsible for cramps and discomfort in the abdominal region 3. Several strategies are used in this treatment, both in modern and traditional medicine, using medicinal plants. In scientific terms, ethnobotanical, biochemical, pharmacological, and toxicological studies and clinical trials have been conducted to provide evidence of the use of plant drugs 4-6. To this end, among the plant species widely used for their medicinal properties is Diospyros mespiliformis Hochst. ex A. DC (Ebenaceae) 6. Studies carried out on this plant have demonstrated the anti-proliferative properties of trunk bark extracts 7 and the antioxidant and antimicrobial activities of organic leaf extracts 8. The anti-

Phytochemical profile, acute oral toxicity, antioxidant, and antispasmodic effects of ethyl acetate and aqueous residual fractions of Diospyros mespiliformis Hochst. ex A. DC (Ebenaceae) leaves on isolated duodenum of rat

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Abstract

Introduction: Diospyros mespiliformis Hochst. ex A. Rich (Ebenaceae) is a nutritional, artisanal and medicinal plant. It is used in alternative medicine in Burkina Faso for the treatment of conjunctivitis, menorrhagia, dysentery, and especially diarrhea. Aims: Our study aimed to evaluate the chemical profile, the antioxidant and anti-inflammatory activities, the safety of use, and the spasmyloptic effects of the fractions obtained from the aqueous decoction of the leaves of Diospyros mespiliformis. Methods: Phytochemical screening by HPTLC and the determination of compounds of interest were carried out. The antioxidant activity was evaluated according to the ABTS, DPPH, FRAP, and LPO methods. The inhibitory activity of Phospholipase A2 and 15-lipoxygenase was evaluated. Acute oral toxicity was carried out on female mice (NMRI). The ex vivo spasmyloptic effect of the fractions was tested on isolated rat duodenum using ACh and BaCl2 as contracting agents. Results: At the end of these tests, the fractions contain flavonoids, tannins, sterols, triterpenes, and saponosides. The content of total phenolics was respectively for the ethyl acetate fraction (EAF) and the residual aqueous fraction (RAF) 84.15±1.73 mg EAA/g and 89.67±2.35 mg EAT/g. That of flavonoids was respectively 45.91±0.98 mg EQ/g and 50.46±0.28 mg EQ/g for the two fractions. The 50% inhibitory concentration (IC50) of EAF for the ABTS, DPPH, FRAP, and LPO methods. The inhibitory activity of Phospholipase A2 compared to Betamethasone. Finally, the most active EAF caused a spasmyloptic effect with Emax of 87.4±15.7% and 1136.25±2.45%, respectively, during contractions induced by BaCl2 and ACh. Conclusion: Finally, this work provided scientific data and could justify the use of Diospyros mespiliformis leaves in the treatment of diarrhea.

Keywords: Diospyros mespiliformis, Leaves, Antioxidants, Anti-inflammatory, Safety of use, Spasmyloptic

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plasmoidal activity of Diospyros mespiliformis trunk bark and leaf extracts on Plasmodium berghei has also been demonstrated. The inhibition of the α-glucosidase enzyme by bioactive compounds isolated from Diospyros mespiliformis has been documented. Preliminary studies have shown that the aqueous decoction of the plant's leaves has spasmylytic effects. However, the antispasmodic properties of leaf fractions have not yet been elucidated. It was therefore essential to assess the safety and spasmylytic efficacy of the ethyl acetate and aqueous residual fractions of Diospyros mespiliformis leaves.

**MATERIAL AND METHODS**

**Chemicals and Reagents**

Chloroform, Ethyl acetate, Methanol, formic acid, Hexane, Dimethyl sulfoxide (DMSO), NEU reagent, aluminum trichloride, iron chloride, ferric trichloride, Folin Ciocalteu reagent (FCR), sulphuric anisaldehyde reagent, Liebermann and Burchard reagent, monobasic potassium phosphate, sodium phosphate dibasic, 15-lipoxygenase (EC 1.13.11.12), linoleic acid, sodium bicarbonate, potassium hexacyanoferrate, trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide solution, 2,2'-azino-bis-[3-ethylbenzothiazoline-6-sulphonique] (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and potassium persulfate were purchased from Sigma Aldrich (St. Louis, MO, USA). Gallic acid, Quercetin, Ascorbic acid, Trolox, Betamethasone, and Zileuton were supplied by Sigma Aldrich. Silica gel TLC plates F 254 grade was from Macherey-Nagel (Germany).

**Plant material**

Fresh leaves of Diospyros mespiliformis Hochst. ex A. DC (Ebenaceae) were collected in May 2022 in Goundi, in the Sanguié Province of Burkina Faso. A botanist from the Laboratoire de biologie végétale et d’écologie de l’Université Joseph Ki-ZERBO, Burkina Faso, identified and authenticated a sample. A specimen exists under number 4267 (OUA). The leaves were dried in the shade, away from direct sunlight and left for 14 days in a thermostated oven at 40 °C. The dried leaves were ground into a fine powder and used for the various tests.

**Preparation of the plant material**

One hundred (100) g of the plant powder was dissolved in 500 mL of distilled water and boiled for 30 min. After cooling, the supernatant was filtered through a fine mesh nylon cloth and centrifuged at 2000 rpm for 5 min to give the aqueous decoctate. The decoctate was subjected to liquid-liquid fractionation with ethyl acetate. This organic phase was concentrated in a rotavapor at 70 °C and oven-dried at 60 °C to give the ethyl acetate fraction, EAF (0.85%). The residual aqueous phase was freeze-dried to give the residual aqueous fraction, RAF (11.33%). These 2 fractions were used for the various tests.

**Phytochemical Investigation: High-performance thin-layer chromatography**

High-performance thin-layer chromatography (HPTLC) was used to detect flavonoids and tannins in the two fractions (EAF and RAF). It was carried out on chromatoplates (60 F 254, 10 x 5 cm, glass support 10 x 20 cm, Merck) following the literature. Approximately 20 μL of each extract was streaked with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 0.8 cm from the bottom edge of the plate. After deposition and drying, the plates were placed in a tank containing eluent previously saturated (2 x 1 dm, saturation time: 30 min). The solvent system used depended on the metabolite to be identified: ethyl acetate/formic acid/H₂O, (8/2/1 v/v/v) for flavonoids; ethyl acetate/formic acid/H₂O (18/2/4/2/1 v/v/v/v/v) for tannins; ethyl acetate/hexane (8/2 v/v) for sterol-triterpenes and hexane/ethyl acetate/methanol (18/5/5 v/v/v/v) for saponosides. After migration over 8 cm in length, the plates were dried, and Neuf reagent for flavonoids, sulphuric anisaldehyde reagent for saponosides, Liebermann and Burchard reagent for Sterol-triterpenes and 5% FeCl₃ for tannins revealed the chromatographic profiles. The profiles were then observed under visible light (tannins) and at UV wavelengths of 366 nm.

**Experimental animals**

Female NMRI (Naval Medicinal Research Institute) mice and Wistar male rats with average weights of 27 ± 4 g and 185 ± 23 g, respectively from the animal house of the “Institut de Recherche en Sciences de la Santé/Centre National de Recherche Scientifique et Technologique (IRSS/CNRST), Burkina Faso” were used. The animals were placed in an enclosure at a temperature of 21-23 °C with a relative humidity of 55 ± 5% and subjected to the light/dark cycle of 12 h/12 h according to the rearing conditions of these species. Standard laboratory pellets enriched with proteins (29%) and water were provided for satiation and experiments were carried out following the procedures of the Guide of Good Practices in Animal Experimentation under the Declaration of Helsinki. Furthermore, all experimental animal procedures have been performed by the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments.

**Spasmylytic effect of two fractions on isolated rat duodenum**

The protocol used has been described by Boly et al. Wistar rat is fasted for 24 h before the start of the experiment and then sacrificed. A portion of the duodenum is removed and immediately placed in Tyrode’s oxygenated physiological solution [KCl (0.2 g), NaCl (8 g), MgCl₂ (0.01 g), NaHCO₃ (1 g), NaH₂PO₄ (0.05 g), CaCl₂ (0.2 g), and Glucose (1 g) in 1 L of distilled water]. A 15 mm fragment was freed of adhesions and mounted in the isolated organ bath thermostated at 37 °C, with a pneumatic bubbler for organ oxygenation. One end of the isolated intestine fragment is attached to the hook of the support rod, and the other end to the isometric transducer, which in turn is connected to the recorder via an amplifier. This device visualizes the contractions of the isolated rat duodenum. The Tyrode solution is renewed every 15 min during the 45 min stabilization period. After observing the regularity of the contractile activity of the isolated organ, KCl (80 mM) is administered into the vessel to stimulate the organ, followed by rinsing. Solutions of the fractions (EAF and RAF) are administered on the one hand, and on the other, after precontraction with acetylcholine (ACH, 10⁻⁶ M) or barium chloride (BaCl₂, 160 μg/mL). This makes it possible to assess, respectively, the extract’s effect on normal contractile activity in the isolated intestine and its interaction with the cholinergic system and potassium fluxes in the cells. The percentage inhibition of contraction (PI) is calculated using the following formula:

\[ PI = \left(1 - \frac{h \text{1}}{h \text{2}} \right) \times 100 \]

h1: height of peaks due to contractor alone; h2: height of peaks due to contractor in the presence of extract.

**Statistical analysis**

Values are given as arithmetic means ± SEM. The significance of differences between means was conducted by GraphPad Prism in version 8.4.3. Student’s t-test, one- and two-way ANOVA, followed by Bonferroni multiple comparison tests were used for comparisons. The difference was considered statistically significant for a threshold of p-value < 0.05.
RESULTS

HPTLC phytochemical investigation

The phytochemical analysis of EAF and RAF highlighted the presence of saponosides, tannins, flavonoids, and sterol-triterpenes (Figure 1).

![HPTLC phytochemical profile](image)

**Figure 1:** Phytochemical profile of ethyl acetate fraction (EAF), and residual aqueous fraction (RAF) revealed by HPTLC

**Total phenolic and flavonoid contents in *D. mespiliformis* fractions**

Table I: Total phenolic and flavonoid content of ethyl acetate and residual aqueous fractions

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenolics (mg TAE/g)</th>
<th>Flavonoids (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAF</td>
<td>84.15 ± 1.73</td>
<td>45.91 ± 0.98</td>
</tr>
<tr>
<td>RAF</td>
<td>89.67 ± 2.35</td>
<td>10.46 ± 0.28***</td>
</tr>
</tbody>
</table>

QE: quercetin equivalent; TAE: tannic acid equivalent; ***p < 0.001 vs EAF

The total phenolic and flavonoid contents are shown in Table I. The two fractions showed the similar value of total phenolic compound content and Ethyl acetate fraction had a high flavonoid compound content.

**Biological activities**

**Antioxidant activity**

The antioxidant activity of *Diospyros mespiliformis* leaves fractions is shown in Table II. EAF had an IC$_{50}$ of 2.26±0.16 µg/mL using the ABTS test. This was statistically significant compared to Trolox (3.78±0.21 µg/mL). For the DPPH radical reduction method, the IC$_{50}$ were 22.34±7.33 µg/mL and 18.58±2.91 µg/mL respectively for EAF and RAF. Significance was obtained between the IC$_{50}$ of these two fractions and the reference compound (Trolox, 6.34±0.04 µg/mL). The ferric ion reduction capacity (FRAP) was 1136.25±0.90 mol EAA/g (EAF) and 1138.4±1.27 mol EAA/g (RAF). The lipid peroxidation inhibitory power (LPO), expressed as a percentage (%) (at 100 µg/mL) was 43.80±6.31% for the EAF, 45.14±10.35% for RAF and 48.11±3.88% for Trolox.

Table II: *In vitro* antioxidant activity of *Diospyros mespiliformis* leaves fractions

<table>
<thead>
<tr>
<th>Extracts</th>
<th>ABTS IC$_{50}$ (µg/mL)</th>
<th>ABTS ARP</th>
<th>DPPH IC$_{50}$ (µg/mL)</th>
<th>DPPH ARP</th>
<th>FRAP mol EAA/g</th>
<th>LPO Inhibition (%) (at 100 µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAF</td>
<td>2.26±0.16*</td>
<td>0.44</td>
<td>22.34±7.23**</td>
<td>0.045</td>
<td>1136.25±0.90</td>
<td>43.80±6.31</td>
</tr>
<tr>
<td>RAF</td>
<td>16.82±0.23***</td>
<td>0.059</td>
<td>18.58±2.91*</td>
<td>0.054</td>
<td>1138.4±1.27</td>
<td>45.14±10.35</td>
</tr>
<tr>
<td>Trolox</td>
<td>3.78±0.21</td>
<td>0.26</td>
<td>6.34±0.04</td>
<td>0.16</td>
<td>48.11±3.88</td>
<td></td>
</tr>
</tbody>
</table>

EAF: ethyl acetate fraction; RAF: Residual aqueous fraction; IC$_{50}$ inhibition concentration 50%; ARP: anti-free radical power; n = 3; *p < 0.05; ***p < 0.001 vs Trolox for ABTS, DPPH, and LPO; EAA: Ascorbic acid equivalent.
**In Vitro Anti-inflammatory activity**

The evaluation of the *in vitro* anti-inflammatory activity of the two leaves fractions by inhibiting 15-lipoxygenase and Phospholipase A2 is recorded in Table III. EAF, and RAF have similar effects in terms of inhibition on 15-lipoxygenase. However, the Zileuton presented a better IC50, 2.92±0.32 µg/mL (***p < 0.001). The evaluation of the effect of fractions on the activity of Phospholipase A2 expressed as a percentage of inhibition shows that there was no statistical difference between EAF, RAF, and Betamethasone (reference substance).

Table III: 15-Lipoxygenase and Phospholipase A2 inhibitory activity of Diospyros mespiliformis leaves fractions

<table>
<thead>
<tr>
<th>Fractions</th>
<th>15-Lipoxygenase IC50 (µg/mL)</th>
<th>Phospholipase A2 Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAF</td>
<td>13.40±1.02***</td>
<td>37.53±1.92</td>
</tr>
<tr>
<td>RAF</td>
<td>13.08±1.46***</td>
<td>30.94±4.90</td>
</tr>
<tr>
<td>Zileuton</td>
<td>2.92±0.32</td>
<td>----</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>----</td>
<td>35.39±3.31</td>
</tr>
</tbody>
</table>

n = 3; ***p < 0.001 vs Zileuton

**Acute oral toxicity**

The dose of 2000 mg/kg body weight (bw) showed no signs of mortality or remarkable behavioral changes in female mice at the first and second stages of administration of the residual aqueous fraction. As for EAF, the dose of 300 mg/kg did not cause mortality during the two-administration series (Table IV).

Table IV: Mortality of female mice administered a single dose of fractions from D. mespiliformis leaves

<table>
<thead>
<tr>
<th>Fractions administered</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st test</td>
</tr>
<tr>
<td>Control (1% Tween 80)</td>
<td>0/3</td>
</tr>
<tr>
<td>EAF (2000 mg/kg)</td>
<td>3/3</td>
</tr>
<tr>
<td>EAF (300 mg/kg)</td>
<td>0/3</td>
</tr>
<tr>
<td>RAF (2000 mg/kg)</td>
<td>0/3</td>
</tr>
<tr>
<td>Excitement</td>
<td>--</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>--</td>
</tr>
<tr>
<td>Hair standing up</td>
<td>--</td>
</tr>
<tr>
<td>Lack of appetite</td>
<td>--</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>--</td>
</tr>
<tr>
<td>Vomiting</td>
<td>--</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>--</td>
</tr>
</tbody>
</table>

**Changes in body weight, food, and water consumption of mice after 14 days of monitoring**

Figure 2 shows the mean weight gain, feed consumption (g/g of mice), and water consumption (mL/g of mice) for 14 days in female mice given a vehicle (1% Tween 80, 10 mL/kg), a single dose (2000 mg/kg) of RAF, and 300 mg/kg of EAF. There was no statistically significant difference in body weight gain between the treated and control batches.

Figure 2: Changes in weight (A) and feed (B) and water (C) consumption of female mice from control and test batches with D. mespiliformis leaves fractions during 14 days of follow-up; n = 6
Macroscopic observation and relative organ weights of mice

Fresh macroscopic examination of vital organs such as the heart, lungs, liver, kidneys, and spleen of control mice and mice treated with EAF and RAF showed that there were no lesions, nor any change in color or appearance of the various organs. Table V shows the relative organ weights of batches of control mice and mice treated with EAF (300 mg/kg) or RAF (2000 mg/kg). No statistically significant variation was observed between the relative organ weights of control and treated batches.

Table V: Relative weights of female mice from control and test batches with *D. mespiliformis* leaves fractions during 14 days of follow-up; n = 6

<table>
<thead>
<tr>
<th>Substances</th>
<th>Heart (mean±SD)</th>
<th>Kidneys (mean±SD)</th>
<th>Lungs (mean±SD)</th>
<th>Liver (mean±SD)</th>
<th>Spleen (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Tween 80, 1%)</td>
<td>0.52±0.03</td>
<td>1.17±0.03</td>
<td>0.80±0.04</td>
<td>5.08±0.3</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>EAF (300 mg/kg)</td>
<td>0.51±0.02</td>
<td>1.20±0.05</td>
<td>0.83±0.03</td>
<td>5.08±0.2</td>
<td>0.44±0.03</td>
</tr>
<tr>
<td>RAF (2000 mg/kg)</td>
<td>0.53±0.06</td>
<td>1.22±0.04</td>
<td>0.79±0.06</td>
<td>5.15±0.16</td>
<td>0.45±0.03</td>
</tr>
</tbody>
</table>

EAF: Ethyl acetate fraction; RAF: Residual aqueous fraction

Antispasmodic effect of EAF and RAF of *D. mespiliformis* leaves on isolated rat duodenum

The results of the relaxation of the isolated rat duodenum the two fractions (EAF and RAF) of *D. mespiliformis* leaves and vehicle are presented in Figure 3. Figure 3A shows the relaxation curves for both fractions and the control on the isolated duodenum precontracted with ACh. The EAF curve was more deviated to the left compared to RAF and control. The histogram (figure 3B) shows the Emax of EAF (90.40±7.84%), RAF (40.21±12.79%) and control (0.88±0.42%). A statistically significant difference was noted between the effects of the two fractions compared with the control. Figure 4A shows similar relaxation results obtained with the fractions and the vehicle (control) on the isolated rat duodenum pre-contracted with BaCl2. With a statistically significant difference, Figure 4B presented the Emax of the EAF (87.4±15.71%) and RAF (51.8±12.88%) fractions and the control (10.9±7.48%).

**Figure 3:** Relaxation curves for ethyl acetate (EAF) and residual aqueous (RAF) fractions of *D. mespiliformis* leaves on isolated ACh-precontracted rat duodenum (A) and histogram of maximum relaxation effect (B); n = 5; ***p < 0.001 vs Control

**Figure 4:** Relaxation curves for ethyl acetate (EAF) and residual aqueous (RAF) fractions of *D. mespiliformis* leaves on isolated BaCl2-precontracted rat duodenum (A) and histogram of maximum relaxation effect (B); n = 5; ***p < 0.001 vs Control
DISCUSSION

Traditional herbal medicine has been used to treat illnesses since ancient times. This practice is used to treat gastrointestinal disorders 15. As such, the leaves of Diospyros mespiliformis are widely used as an antispasmodic 16. The aim of this study was to provide scientific data on the use of the plant in the treatment of diarrhoea in alternative medicine. The phytochemical screening of Diospyros mespiliformis leaves fractions is comparable to that reported in studies showing that D. mespiliformis leaves contain flavonoids, tannins, steroids, terpenes and saponosides 6, 17. These phytochemicals neutralise reactive oxygen species and superoxides, while other flavonoids can trap the highly reactive oxygen radical known as peroxynitrite 18. Flavonoids have anti-inflammatory and antispasmodic properties. They also have anti-inflammatory, anti-diarrhoeal, anti-parasitic and antibacterial properties 16. In addition, total phenolic content was measured in the ethyl acetate fraction (84.15±1.73 mg TAE/g) and the residual aqueous fraction (89.67±2.35 mg TAE/g). Higher levels of total phenolic compounds in aqueous, ethanolic, methanolic and petroleum ether extracts of Diospyros mespiliformis leaves have been demonstrated 6, 18. These compounds are known for their spasmolytic properties 19. In addition, phenolic compounds, in particular flavonoids, tannins and triterpenes, are inhibitors of certain pro-inflammatory enzymes, chelators of heavy metals involved in the production of free radicals and the regulation or protection of the antioxidant defense system 20, 21. The antioxidant properties of D. mespiliformis leaves extracts have already been demonstrated 6, 22, 23. Moreover, in the spasmodic mechanism, inflammation of the viscerina cannot be ignored, hence the search for anti-inflammatory properties in the fractions. Overall, the fractions showed good inhibition of phospholipase A2 and 15-lipooxygenase. These anti-inflammatory activities were less effective than Zileuton, but similar to Betamethasone. These results confirm the anti-inflammatory properties of the plant’s leaf fractions 23. The flavonoids and sterols/triterpenes in the fractions are known for their ability to inhibit pro-inflammatory enzymes 13, 24. In addition, for the safe use of both fractions, acute oral toxicity was assessed. The results showed that acute oral administration of the ethyl acetate fraction (2000 mg/kg bw) resulted in mortality in mice. This finding indicates that EAF should be used sparingly. However, EAF (300 mg/kg bw) and RAF (2000 mg/kg bw) did not cause any mortality or behavioural changes. Thus, the LD₅₀ of these two fractions was estimated at 1000 mg/kg and 5000 mg/kg bw respectively for EAF and RAF according to the United Nations Globally Harmonised System 25. These results suggest that at very high doses, AEF can have harmful effects on consumers. In line with the literature, work has shown that the methanolic extract of the leaves and bark of the trunk of Diospyros mespiliformis, as well as their hexane, ethyl acetate and butanol fractions, can be safely consumed 26. Pharmacological results showed that EAF and RAF have muscle relaxant properties on isolated rat duodenum after stimulation of acetylcholine receptors by ACh with best efficacy for RAF. ACh induces a significant positive tonotrop effect marked by a contracture with plateau contractile activity reflecting the increased peristalsis of the gastrointestinal tract. ACh induces contraction through the activation of G protein-coupled smooth muscle M₃ receptors, leading via inositol Triphosphate (IP₃) to the release of intracellular Ca²⁺ 27, 28. This curative experiment shows that EAF and RAF have anticholinergic properties. This property can be explained by the presence of tannins, flavonoids, saponosides and terpenoids in the fractions, which block the action of ACh. Indeed, these compounds are known for their anti-diarrhoeal properties through their spasmodic effects 19, 29. Moreover, these results are in agreement with the literature, which has documented the anti-diarrhoeal effects of a decoction of D. mespiliformis leaves, traditionally used in Ghana and Nigeria 8. Preliminary results with the freeze-dried aqueous decoctate of the plant’s leaves not documented in the present work also showed spasmodic effects on isolated rat duodenum. More effective than EAF but weaker than RAF. The cytoplasmic increase in Ca²⁺ concentration in smooth muscle cells is the main stimulus for contraction, which usually results from both intracellular release of stored Ca²⁺ and influx of extracellular Ca²⁺ 30. The concentration-dependent spasmodic effect of EAF and RAF on contractile activity in the isolated gut could be the result of Ca²⁺ uptake by phosphorylated proteins under the influence of cAMP-activated protein kinase. It may also be due to an inhibition of calcium influx or an increase in calcium efflux without altering influx, causing gastrointestinal smooth muscle relaxation 31, 32. In addition, RAF and RAF inhibit BaCl₂-induced smooth muscle contraction. Indeed, both fractions at concentrations of 0.03-10 mg/mL induced a relaxant effect on the rat duodenum by significatively and concentration-dependently reducing contractions. This effect could be due to an action similar to papaverine with a musculotropic effect, by inhibiting phosphodiesterase function 14. However, the mechanism of action of these fractions needs to be studied in greater depth, especially the assessment of their in vivo anti-diarrhoeal properties and their medium- and long-term oral toxicity.

CONCLUSION

We are not aware of any work on the spasmytic properties of Diospyros mespiliformis leaves fractions. Results have shown that ethyl acetate and residual aqueous fractions of Diospyros mespiliformis leaves inhibit the contractile actions of intestinal muscle by ACh and BaCl₂. These effects are thought to be mediated by tannins, saponosides, flavonoids, sterols and terpenoids, which also have antioxidant and anti-inflammatory effects. The ethyl acetate fraction was moderately toxic at high doses compared to the residual aqueous fraction. Thus, the present study contributes to new knowledge of the spasmytic effects of Diospyros mespiliformis leaves on the isolated rat duodenum, and reinforces the traditional use of this plant for gastrointestinal symptoms. However, further research is needed to improve our understanding of the mechanisms involved.

CONFLICT OF INTERESTS

Authors have declared that no competing interests exist.

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Authors have declared that no competing interests exist.

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3. Heghes SC, Vostinaru O, Rus LM, Mogosan C, Iuga CA, Filip L. The ethyl acetate and residual aqueous fractions of Diospyros mespiliformis leaves inhibit the contractile actions of intestinal muscle by ACh and BaCl₂. These effects are thought to be mediated by tannins, saponosides, flavonoids, sterols and terpenoids, which also have antioxidant and anti-inflammatory effects. The ethyl acetate fraction was moderately toxic at high doses compared to the residual aqueous fraction. Thus, the present study contributes to new knowledge of the spasmytic effects of Diospyros mespiliformis leaves on the isolated rat duodenum, and reinforces the traditional use of this plant for gastrointestinal symptoms. However, further research is needed to improve our understanding of the mechanisms involved.