Gastroprotective activity of the resin from *Virola oleifera*

Ana Claudia Hertel Pereira\textsuperscript{a}, Dominik Lenz\textsuperscript{a}, Breno Valentim Nogueira\textsuperscript{b}, Rodrigo Scherer\textsuperscript{a}, Tadeu Uggere Andrade\textsuperscript{a}, Helber Barcellos da Costa\textsuperscript{b}, Wanderson Romão\textsuperscript{b,c}, Thiago Melo Costa Pereira\textsuperscript{a,c}\textsuperscript{*} and Denise Coutinho Endringer\textsuperscript{a,c}\textsuperscript{*}

\textsuperscript{a}Pharmaceutical Sciences Graduated Program, University Vila Velha, Vila Velha, Brazil; \textsuperscript{b}Morphology Department, Federal University of Espírito Santo, Vitória, Brasil; \textsuperscript{c}Federal Institute of Education, Science and Technology (IFES), Vila Velha, Brazil

\textbf{ABSTRACT}

\textbf{Context:} The resin from the trunk wood of *Virola oleifera* (Schott) A. C. Smith (Myristicaceae) is used in folk medicine to hasten wound repair and to treat pain and inflammatory conditions, and our previous report indicated the anti-oxidative properties in other oxidative stress model.

\textbf{Objective:} To investigate the protective effects of resin from *V. oleifera* in two experimental models of gastric ulcer oxidative-stress dependent.

\textbf{Materials and methods:} Plant material was collected and the resin was subjected to partitioning with organic solvents. The butanol fraction was subjected to chromatographic and spectrometric methods for isolation and structural elucidation. The resin was quantified for polyphenols and flavonoids by colorimetric methods. Furthermore, the antioxidant activity of resin was determined by three different methods. The ulcers were induced acutely in Swiss male mice with ethanol/HCl and indomethacin using single-doses of 10 and 100 mg/kg. The gastroprotection of the experimental groups was comparable to reference control lansoprazole (3 mg/kg).

\textbf{Results:} The high content of polyphenols (~82%) and the presence of epicatechin and eriodictyol were determined. The LD\textsubscript{50} was estimated at 2500 mg/kg. At minimum (10 mg/kg) and maximum (100 mg/kg) dosage of resin, both in ethanol/HCl as indomethacin ulcer induction models demonstrate reduction of lesions (minimum: ~97% and ~66%; maximum: ~95% and ~59%).

\textbf{Discussion:} The gastroprotection might be related to tannins, phenolic acids and flavonoids present in the resin by antioxidant properties.

\textbf{Conclusions:} The results indicate that this resin has gastroprotective activity probably associated with the presence of phenolic antioxidant substances.

\section*{Introduction}

*Virola oleifera* (Schott) A. C. Smith (Myristicaceae), commonly known as ‘bicuíba’, ‘bocuva’, ‘bicuíva’, ‘ucuiba’, ‘candêia-do-caboclo’, is a tree of the Atlantic Forest (Colombo & Joly 2010). The oil extracted from seeds of *Virola* species is popularly used for rheumatic pains, bronchial asthma, tumors in the joints, intestinal worms, halitosis, haemorrhoids and skin diseases (Rodrigues 1980). The bark when grated produces a resin that is used against chronic wounds, diarrhoea, leucorrhoea and haemoptysis (Rodrígues 1980; Böa et al. 2015). Despite its widespread use in popular medicine against a myriad of diseases, there were no scientific reports in literature supporting the use of crude resin as an anti-ulcer agent.

The phytochemical analysis performed in this study determined the content of phenolic substances, as there are reports about the gastroprotective properties of these substances (Mota et al. 2009; Bansal & Goel 2012), and they have also been identified in several species of *Virola* bark (Hiruma-Lima et al. 2009) among other plants (Thirunavukkarasu et al. 2009; Alimi et al. 2010). Recently, data from our laboratory (Böa et al. 2015) demonstrated the presence of phenolic acids in the resin from *V. oleifera* and an antioxidant effect in vitro and in vivo.

The hypothesis is that the resin of *V. oleifera* has gastroprotective activity, due to the presence of flavonoids and phenolic compounds. Therefore, the aim of this study was to characterize the chemical resin of the plant *Virola oleifera*, existing in the Atlantic Forest in the state of Espírito Santo, and evaluate its antioxidant and gastroprotective effect. In the search of new potential analgesic/anti-inflammatory agents with gastroprotective properties, the present study evaluated its effects in experimental models of gastric ulcer.

\section*{Materials and methods}

\textbf{Animals}

Male Swiss mice *Mus musculus* (25–40 g) from the Experimental Monitoring Laboratory of University Vila Velha (UVV) were used in the experiments. The animals were fed a normal chow diet and water ad libitum under standard conditions of...

\begin{figure}

\caption{Gastroprotective activity of the resin from *Virola oleifera*}

\end{figure}
dark–light cycle of 12 h and temperature (23 ± 3 °C). Prior to all gastroprotective assays the animals were 24 h fasting, with access only to 10% glucose solution ad libitum. All biological assays were approved by Ethics, Bioethics and Animal Welfare of UVV (CEUA-UVV 150/2011) and were performed according to the international principles accepted from NIH 85-23.

**Chemicals and reagents**

Pyrogallol (≥99%), quercetin (≥98%), butylhydroxytoluene (BHT), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Potassium persulfate, sodium acetate trihydrated, TPTZ and iron chloride were supplied from VETEC Quimica Fina LTDA. Lansoprazole were purchased from Henrifarma® (Henrifarma Produtos Químicos e Farmacêuticos LTDA, São Paulo-Brazil). Other reagents were of HPLC grade or analytical grade. Milli-Q water (Millipore, Missouri, NH) was used throughout this study, and all solutions were prepared immediately before use.

**Resin material**

The resin of Virola oleifera (Schott) A. C. Smith (Myristicaceae) was collected in February 2012 from the district of Fazenda Guandu, in Afonso Claudio (Espírito Santo – Brazil, S 20° 13490′ W 041° 06692′). The resin was collected with authorization (IEMA 6 2 9/09) and in accordance with the Brazilian law (Resolution 29, 12/06/2007), which states that no special permission is necessary to collect samples of essential oil or fixed oil, or when the tested material remains similar to the raw material (Provisional Statement 2.186-16, 08/23/2001). The plant material was verified by D.Sc. Luciana Dias Thomaz, Department of Botany, Federal University of Espírito Santo, where the voucher specimen was deposited (VIIES 19648). The fluid exudate was obtained from 0.5 cm deep incisions in the tree trunk. The resin was collected in aseptic plastic containers and transferred to an amber glass vial and was kept in +4 °C until the analysis. The fluid exudate was then subjected to drying at 40 °C. After drying, it was grained and 24 g of dried resin was obtained.

**Isolation and identification of substances**

An aliquot of the dried resin (10 g) was re-suspended in water and partitioned with dichloromethane (3 × 20 mL), followed by buthan-1-ol (3 × 20 mL). The fractions were concentrated to residue, providing 0.0047 g of dichloromethane fraction (VOD), 1.06 g of buthanol fraction (VOB) and 7.91 g of the aqueous fraction (VOA). The butanol fraction (1.0 g) was re-suspended in methanol and applied on a column of Sephadex LH-20 (19 cm × 2 cm, 6 g, Sigma Aldrich) using HPLC grade methanol as an eluent. Fractions (10 mL) were collected and analyzed by TLC [Si gel plates, ethyl acetate:formic acid:water (18:1:2)] and grouped, resulting in 22 fractions (F1–F22).

The F7, which was tested positive for polyphenols in the presence of ferric chloride (FeCl3, 1%), was dried and rechromatographed on silica gel column 60 M (Macherey Nagel 0.04–0.0063 mm; 230–400 mesh) (24 cm × 2.5 cm, 15 g), eluting first with ethyl acetate:formic acid:water (18:1:2) and gradually decreasing the percentage of ethyl acetate until its complete removal (Sun et al. 2007). Fractions (10 mL) were collected and analyzed by TLC [ethyl acetate:formic acid:water (18:1:2)], resulting in a total of 15 fractions (F7-1 to F7-15). The fraction F7-2 and F7-3 that had showed positive result with FeCl3, with visualization of a single band, underwent spectrometric analysis (ESI-MS and NMR).

**Identification of substances by ultra-high resolution and accuracy mass spectrometry (ESI(−)-FT-ICR MS, ESI(−)-IMS/MS)**

The experiments were performed on an ultra-high resolution and accuracy mass spectrometer (model 9.4 T Solaris, Bruker Daltonics, Bremen, Germany). ESI(−)-FT-ICR MS. The ESI(−)-FT-ICR spectrum was acquired in the m/z region of 200–2000. Briefly, the sample was dissolved in a methanol/ammonium hydroxide mixture (99.9/0.1 v/v %), thus resulting in a final concentration of 1 µg/mL. The parameters of ESI(−) source were: (a) capillary voltage: +3000–3500 V; (b) end plate offset = −100 V; (c) temperature and gas flow drying: 180 °C e 4 L/min; and (d) nebulizer gas pressure: 0.5 bar. The ion accumulation time in the hexapole for 0.01 s was followed by transporting to the analyzer cell through the electrostatic lens system. Each spectrum was acquired by accumulating 100 scans of time-erosing transient signals in the length of 4 mega-point time-domain data sets. A resolving power (m/Δm50%) ≈ 500,000, in which Δm50% is the full peak width at half-maximum peak height) of m/z 400 and a mass accuracy of <1 ppm provided unambiguous molecular formula assignments for singly charged molecular ions (Colati et al. 2013). Additionally, tandem mass spectrometry experiments (ESI(−)-FT-ICR MS/MS) were also performed for the ions of m/z 289, 579 and 869. ESI(−)-FT-ICR MS/MS spectra were acquired with an isolation window of 1.0 (m/z units) and 19–27 V of collision energy. All spectra were processed using software package Compass Data Analysis (Bruker Daltonics, Bremen, Germany). The DBE was calculated according to the formula DBE = c – h/2 + n/2 + 1, where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively, in the molecular formula.

**Spectrophotometric quantification of total of polyphenols and flavonoids and tannins**

The determination of total polyphenols, tannins and total flavonoids in the resin were performed as described by Krepsky et al. (2012). The quantification of total polyphenols was expressed as a percentage calculated as pyrogallol equivalent and total as quercetin equivalent.

**Determination of antioxidant activity**

**ABTS Method**

The antioxidant activity of the resin was determined by the method of radical scavenging using ABTS according to Re et al. (1999) with slight modifications. Initially, the radical ABTS⁺ was formed by the mixture of 7.0 mM of ABTS (ethanol 50%) with 2.45 mM of potassium persulfate (deionized water). This reagent was kept under +4 °C for at least 16 h. Before use, the reagent was diluted in ethanol 50% until an absorbance of 1.0 (± 0.01) in 734 nm was obtained. In 96-well microplates, 270 µL of ABTS⁺ radical and 30 µL of each concentration of the compounds were added. In blank well, 30 µL of ethanol was added. After 6 min of reaction in the dark, the absorbance was determined in a 734 nm using a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA). The antioxidant activity was expressed in % = (Abs1 – Abs0) × 100, where Abs0 is the blank
absorbance and Abs1 is the absorbance of the test. The antioxidant activity of the resin was compared with the action of the synthetic antioxidant BHT.

**FRAP method**

The antioxidant activity of the resin was also analyzed by the FRAP (Ferric Reducing Antioxidant Power) method according to Benzie and Strain (1996), with modifications. For the preparation of FRAP reagent, 25 mL of sodium acetate trihydrated (0.3 M; pH 3.6) was mixed to 2.5 mL of TPTZ solution (10 mM) in HCl 40 mM and 2.5 mL of iron chloride aequous solution (20 mM), in a total of 30 mL of FRAP solution, that was used immediately after preparation. An aliquot of 30 μL of test solutions were added to 270 μL of FRAP reagent. For blank, 30 μL of ethanol was mixed with 270 μL of FRAP reagent. After 5 min of reaction, the absorbance was read in 595 nm using an absorbance microplate reader. The antioxidant activity was express in % = (Abs1 – Abs0) × 100, where Abs0 is the blank absorbance and Abs1 is the absorbance of the test. The antioxidant activity of the resin was compared with the action of the synthetic antioxidant BHT.

**Gastroprotective activity assay**

**HCl/ethanol-induced ulcer**

The anti-ulcerogenic activity of the dried resin was studied by ethanol/HCl-induced gastric ulcer. The experiments were performed as described by Oyagi et al. (2010) with adaptations. Mice were divided into six groups of five animals. Each group had fasted prior to receiving an oral dosage of saline (5 mL/kg), lansoprazole (3 mg/kg) (Olate et al. 2012) or dry resin solubilized in saline (at the dosages of 1, 10, 100 mg/kg) and negative control. After 50 min, all groups were treated orally with 0.2 mL of 0.3 μM HCl/60% ethanol solution (ethanol/HCl) for the induction of gastric ulcer, except the negative control group. Animals were euthanized 1 h after the administration of ethanol/HCl, and the stomachs excised and inflated by 1% formalin injection. The extent of the lesion was measured and the lesion index was expressed as the sum of injured areas divided by the area of the extent of the lesion was measured and the lesion index was formed as described by Oyagi et al. (2010) with adaptations. The stomachs were then stored for histological evaluation.

**Indomethacin-induced ulcer (40 mg/kg)**

The experiment was performed according to the method of Djahanguiri (1969) with modifications. Briefly, gastric lesions were induced with indomethacin (40 mg/kg), dissolved in 1.0 mL of sodium bicarbonate 0.5 mol/L) and administered intraperitoneally to mice after fasting. Solubilized resin (10 and 100 mg/kg), lansoprazole (3 mg/kg) (Olate et al. 2012) and vehicle (saline 5 mL/kg) were administrated orally 30 min before the induction of gastric lesions. Five hours after the induction of ulcers, the animals (n = 5) were anesthetized and euthanized. The stomachs were removed and, subsequently, inflated. Gastric damage was determined as described above.

**Acute oral toxicity assay**

The experiments with male Swiss mice were performed in accordance with the ethical principles established by the Brazilian College of Animal Experimentation (COBEA, 1991). The animals were between 8 and 12 weeks old at the beginning of the experiments, with a mean weight of 25 ± 2 g.

Five days before starting the protocol, the animals were housed in cages, one per cage, with free access to water and food. Throughout the experiment, the animals had free access to food and water, except for the 3–4 h of fasting they were subjected to prior administration of the resin and 1–2 h after administration. After the fasting period of 3–4 h, time (t) = 0, the animals were weighed. The resin was administered as a single dose (2000 mg/kg, as specified by protocol OECD 423) by gavage using a suitable intubation cannula (gavage).

Three animals were used for control group, and three for the test group. The control group received the vehicle (drinking water) orally, in an amount equal to the average volume (0.2 mL) of the compound, both in a single dose. After treatment, all animals were closely observed for 4 h and then daily for 14 days; any behavioural changes that occurred were noted.

Additionally, the weight of each animal was monitored during the experiments and at the end of the 3–4 h fasting period as well as along the 14 days after the administration of the studied dose. After the 14th day, any deaths that occurred were recorded to calculate the LD50, and all of the surviving animals were anesthetized with sodium thiopental 120 mg/kg intraperitoneally, then thoracotomy was performed. Blood was collected by cardiac puncture in the right ventricle and it was added to a tube without the addition of an anticoagulant.

The samples were used for biochemical tests of liver function markers (albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], and γ-glutamyl transferase [GGT]) and renal function markers (creatinine, urea and total protein). The animals were then euthanized and their kidneys and livers were extracted and weighted.

After all of the reported procedures were completed, the animals were frozen and all medical waste was collected according to the required protocol. The results observed in the experiments were recorded for each animal, showing the number of animals used, the number of animals presenting signs of toxicity, the number of animals found dead during the test and the number of animals that were euthanized after 14 days. The description and the time course of the toxic effects and the reversibility and the necropsy findings were also documented.

**Biochemical analyses**

Biochemical analyses were performed using a semi-automatic biochemical analyzer (LabmaxPlanno-Labtest, Minas Gerais, Brazil). The biochemical parameters measured were urea (Labtest® cod. 104.2, Minas Gerais, Brazil, linearity ≥300, sensitivity of 0.051–0.061 mg/dL, colorimetric method, 600 nm), creatinine K (Labtest® cod.96.2, linearity of 0.2–12 mg/dL, sensitivity of 0.583–0.648 mg/dL, colorimetric method, 510 nm), total protein (Labtest® cod.99.1, linearity ≥14 g/dL, sensitivity of 0.017–0.019 g/ dL, colorimetric method, 345 nm), albumin (Labtest® cod.19.1, linearity ≥6 g/dL, sensitivity of 0.0091–0.0099 g/dL, colorimetric method, 630 nm), aspartate aminotransferase (AST, Labtest® cod.109.2, linearity ≥400 U/L, sensitivity of 0.1430–1.943 U/L, colorimetric IFCC method, 340 nm), γ-glutamyltransferase (GGT, Labtest® cod.105.2, linearity ≥700 U/L, modified Szasz method, 405 nm), alanine aminotransferase (ALT, cod.108.2, linearity ≥400 U/L, sensitivity of 0.1469–2.029 U/L, colorimetric UV-IFCC method, 340 nm) and alkaline phosphatase (ALP, Labtest® cod.79.4, linearity ≥1500 U/L, colorimetric assay, 405 nm).
Histological analysis

The stomachs of animals undergoing gastric ulcers were opened from the greater curvature and preserved in a solution of 10% buffered formalin. They were then processed for subsequent embedment. The resulting tissue samples blocks were cut into sections of 2.5 μm. Two slides were prepared from each sample containing three consecutive cuts, one of the slides was stained with hematoxylin–eosin (HE) and the other with HE and periodic acid–Schiff (PAS) for staining structures containing a high proportion of mucins in stomach. Samples were analyzed with an Olympus AX70 microscope system with a digital camera (Zeiss AxioCam ERc5S model, Oberkochen, Germany).

Image analysis

The images were obtained with the aid of Semiprofessional Digital Camera Canon Power Shot SX10IS model in resolution 12 megapixel photographs by way of the short distances (macro mode), without flash and positioned 20 cm from the blade to be photographed. The tissue was placed between two glass sheets and pressed lightly to allow the observation of the entire area inside of the stomach. The images were then analyzed using the free software CellProfiler®, Carpenter Lab (Cambridge, MA).

Statistical analysis

The results of the chemical evaluation were expressed as mean ± standard deviation, while the results of the biological evaluation were expressed as mean ± SEM. Statistical comparisons between the different groups were performed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. The results of the biochemical analyses and animal’s body weight for the acute toxicity assay were expressed as mean ± standard deviation (SD). Data were submitted to analysis of variance (ANOVA); the homoscedasticity was evaluated using the Bartlett test. This fact indicated that the resin of V. oleifera would be classified in category 5, with an estimated median lethal dose (LD50) of 2500 mg/kg according to protocol of test 423 (OECD 2001).

No significant change in body weight or correlation to liver/body weight or kidney/body weight was noticed among the other animals. The biochemical analysis showed no difference between the groups (Table 1). The groups tested also did not present differences in behaviour or eating habits.

In the evaluation of the gastroprotective activity, our study is the first to demonstrate benefits with the resin of V. oleifera in two different experimental models, with equal or better efficacy (respectively) when compared to lansoprazole, a classical proton pump inhibitor. Ethanol/HCl induced intense gastric mucosal damage in the control group of mice that received vehicle alone (Figure 3). Oral treatment of 1, 10, 100 mg/kg of V. oleifera significantly reduced the gastric mucosal damage and quantitative reduction (VB1 = 10.62 ± 4.01 RU; VB10 = 0.89 ± 0.27 RU; VB100 = 1.65 ± 0.61 RU, p < 0.05) compared with the control-treated group (31.89 ± 7.33 RU). The dosages greater than 1 mg/kg showed maximum protective response. The lansoprazole group (3 mg/kg) also demonstrated gastroprotection (8.7 ± 2.2 RU, p < 0.05).

The gastroprotective effect of V. oleifera on indomethacin-induced gastric damage was macroscopically determined in mice (Figure 4). Macroscopic lesions with evident borders in various forms and sizes were dispersed irregularly on all stomach surfaces in the stomach tissue of the control mice that received only indomethacin (4.72 ± 1.71 RU). The mice that received oral treatment of 10 and 100 mg/kg of V. oleifera showed same gastric mucosal protection (VB10 = 1.61 ± 0.99* RU; VB100 = 1.93 ± 1.12* RU, p < 0.05), while the lansoprazole group did not show acute gastroprotection (3.16 ± 1.62 RU).

Additionally, histopathological analysis confirmed that pre-treatment with V. oleifera prevented ethanol/HCl and indomethacin-induced histological damage in the superficial layers of the gastric mucosa with congestion by HE staining. Compared with the negative control group, ethanol/HCl administration induced a disruption of the superficial region with epithelial cell loss and intense oedema formation (Figures 5(a,b), respectively). In this model, histological analysis showed that V. oleifera at the same
dosages prevents the development of gastric ulcer (Figures 5(d–g), respectively).

As the indomethacin model, compared with the negative control group, indomethacin administration induced a disruption of the superficial region (HE stain) with the loss of mucus analyzed by Periodic Acid Schiff stain (Figures 6(a,b)). Histological analysis showed that both dosages (10 and 100 mg/kg) of *V. oleifera* prevented this damage (Figures 6(d,e)).

**Discussion**

Ethanol and HCl quickly infiltrate the gastric mucosa and cause membrane damage, exfoliation of cells, erosion and ulcer formation. Consequently, there is an increase in mucosal permeability together with the release of vasoactive products from leucocytes that can lead to vascular injury, oxidative stress and necrosis (Oyagi et al. 2010). On the other hand, it has been suggested that indomethacin induces gastric damage by inhibiting the release of protective factors like cyclooxygenase-1 (COX-1), prostaglandin E2 (PGE2), bicarbonate and mucus, besides reducing antioxidant mechanisms while increasing oxidant factors (Halici et al. 2005). Independent of original mechanism, both models could be well-accepted as oxidative stress-induced stomach disease, since the mechanism of ulcer induction might be mediated by reactive oxygen species (ROS) (Oyagi et al. 2010).

According to the chemical analyses and the *in vivo* assays observed, the maintenance of activity by *V. oleifera* in the presence of indomethacin may be attributed to an adaptive cytoprotection independent in part on PG pathway. Then, we proposed three main mechanisms of gastroprotection by *V. oleifera*: (1) the antioxidant effect of tannins, phenolic acids and flavonoids; (2) the mechanical protection mainly by tannin content and (3) other regulatory mechanisms, explained below.

Firstly, the antioxidant effect shown by our resin *in vitro* and recently *in vivo* by Bóa et al. (2015) corroborates with other studies that associate gastroprotection in the presence of phenolic
substances and antioxidant effects (Mota et al. 2009; Thirunavukkarasu et al. 2009; Bansal & Goel 2012; Monteforte et al. 2014). We speculate that the polyphenols might protect against peptic ulcer by many pathways such as cytoprotection, re-epithelialization and suppressing oxidative damage, as mentioned by Farzaei et al. (2015). Among the polyphenols, the flavonoids have been extensively studied in relation to gastroprotection (Mota et al. 2009). Moreover, the eriodictyol, isolated in the resin, seems to decrease the oxidative stress and so gastric lesions (Lee 2011). As another example, the quercetin, flavonoid measured on the resin from *V. oleifera* (Boa et al. 2015), also seems to reverse damage caused by the imbalance between redox defense system (Coskun et al. 2004). As discussed by Rios et al. (2002), some procyanidins may limit the oxidative stress, reduce the anti-inflammatory response and by that contributing to decrease the lesion in the mucosa. Moreover, several studies have related the gastroprotective activity of extracts and isolated compounds of catechin or their oligomers to its antioxidant activity (Alimi et al. 2010; Bansal & Goel 2012; Monteforte et al. 2014). Epicatechin, identified in the resin, shows peroxyl radical scavenging activity in vitro (Yilmaz & Toledo 2004) contributing directly to the reduction of oxidative stress.

Secondly, the mechanical protection of the resin may be associated with high tannin content, being capable of complexing with proteins or glycoproteins, causing their precipitation over the mucosa, forming an impenetrable layer to harmful agents (Goldstein & Swain 1965; Rios et al. 2002; Thirunavukkarasu et al. 2009). This hypothesis cannot be ruled out, as all treatments and ulcer agent were orally administered. The capacity of complexing of these substances can also result in inactivation of enzymes and preventing the back-diffusion of acid and digestive enzymes (Goldstein & Swain 1965; de Jesus et al. 2012).
Other substances present in V. oleifera (Bôa et al. 2015) such as gallic acid and ferulic, quercetin and catechin could contribute to mucus preservation, maintaining its integrity (Figure 3).

Additionally, the phenolic compounds are also capable of generating increased formation of new capillaries and fibroblasts (Thirunavukkarasu et al. 2009). The phenolic acids, including ferulic and gallic quantified in V. oleifera (Bôa et al. 2015) are beneficial to mucosal protection (Badary et al. 2006; Laine et al. 2008; Barros et al. 2008, Pal et al. 2010). These substances may inhibit the activity of lipoxigenase, decreasing of inflammatory response (Badary et al. 2006), besides stimulating the PGs biosynthesis (Alanko et al. 1999). Also, with the multifactorial aetiology of gastric ulcers, it is estimated that the gastroprotective action of certain phenolic acids, including ferulic, is related not only to a cytoprotective role but also to the H2 receptor antagonism (Barros et al. 2008). In the literature it is described that gallic acid and quercetin inhibit the release of histamine, and may have

Figure 4. Gastroprotective effect of resin V. oleifera in mice. At the top, typical macroscopic images of the stomachs of mice subjected to injury by indomethacin. The bar graph shows the mean number of lesions in the respective groups. The values are represented as mean ± standard error of the mean. Relative number of lesions compared to negative control group. *p < 0.05 vs. control mice, n = 5.

Figure 5. Stomachs photomicrographs stained with hematoxylin and eosin. (a) Histological section of negative control group. (b) Microscopic images of lesions induced by ethanol/HCl in gastric mucosa pretreated with vehicle. (c) Microscopic image of lesions pretreated with lansoprazole 3 mg/kg, (d) Microscopic image of lesions pretreated with V. oleifera at 1 mg/kg, (e) at 10 mg/kg (f) 100 mg/kg and (g) 250 mg/kg. Scale: 100 μm (top micrograph) and 50 μm (lower micrograph). n = 5.
an antisecretory effect (Kahraman et al. 2003; Kim et al. 2006) reducing the damage on the gastric mucosa (Glavin & Szabo 1992).

**Conclusion**

In summary, gastroprotective effects associated to the resin of *Virola oleifera* could be justified by composition being rich in phenolic compounds. Further studies are needed to be conducted to assess its toxicity and its mechanism of action. Analyses regarding the activity of the various pathways involved in gastric ulcer induction and/or oxidative stress are also suggested.

**Acknowledgements**

We acknowledge University of Vila Velha – UVV for providing the knowledge and structure necessary to accomplish this work.

**Disclosure statement**

The authors report no declarations of interest.

**Funding**

FAPES (Fundação Estadual de Amparo à Pesquisa do Estado do Espírito Santo, 10.13039/501100006182) is acknowledged for financially supporting this work and for the fellowship and University Vila Velha (UVV) for providing the knowledge and structure necessary to accomplish this work.

**References**

Alanko J, Riutta A, Holm P, Mucha I, Vapaatalo H, Metsa-Ketela T. 1999. Modulation of arachidonic acid metabolism by phenols: relation to their structure and antioxidant/prooxidant properties. Free Radical Bio Med. 26:193–201.

Alimi H, Hfaiedh N, Bouoni Z, Hfaiedh M, Sakly M, Zourgui L, Rouma KB. 2010. Antioxidant and antiulcerogenic activities of *Opuntia ficus indica f. inermis* root extract in rats. Phytomedicine. 17:1120–1126.

Badary OA, Awad AS, Sherief MA, Hamada FMA. 2006. *In vitro* and *in vivo* effects of ferulic acid on gastrointestinal motility: inhibition of cisplatin-induced delay in gastric emptying in rats. World J Gastroentero. 12:5363–5367.

Baliano AP, Alves FS, Pereira ACH, Aquije GM, de FV, Lenz D, Andrade TU, Endringer DC. 2015. Centennial knowledge of medicinal plants held in communities of Espirito Santo, Brazil. Ethnobot Res Appl. 14:155–162.

Bansal VK, Goel RK. 2012. Gastroprotective effect of *Acacia nilotica* young seedless pod extract: Role of polyphenolic constituents. Asian Pac J Trop Med. 5:523–528.

Barros MP, Lemos M, Mastro EL, Leite MF, Sousa JPB, Bastos JK, Andrade SF. 2008. Evaluation of antulcer activity of the main phenolic acids found in Brazilian Green Propolis. J Ethnopharmacol. 120:372–377.

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem. 239:70–76.
Colati KAP, Dalmacchio GP, Castro EVR, Gomes AO, Vaz BG, Romão W. 2013. Monitoring the liquid/liquid extraction of naphtenic acids in Brazilian crude oil using electrospray ionization FT-ICR mass spectrometry (ESI FT-ICR MS). Fuel. 108:647–655.

Colombo AF, Joly CA. 2010. Brazilian Atlantic Forest lato sensu: the most ancient Brazilian forest, and a biodiversity hotspot, is highly threatened by climate change. Braz J Biol. 70:697–708.

Coskun O, Kanter M, Armutc¸u F, Çetin K, Kaybolmaz B, Yazgan O. 2004. Protective effects of quercetin, a flavonoid antioxidant, in absolute ethanol-induced acute gastric ulcer. Eur J Gen Med. 1:37–42.

de Jesus NZ, de Souza Falcão H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, Barbosa-Filho JM, Tavares JF, Silva MS, Athayde-Filho PF, Batista LM. 2012. Tannins, peptic ulcers and related mechanisms. Int J Mol Sci. 13:3203–3228.

Bœ JSF, Porto ML, Pereira AC, Ramos JPL, Scherer R, Oliveira JP, Nogueira BV, Meyrelles SS, Vasquez EC, Endringer DC, et al. 2015. Resin from Virola oleifera protects against radiocontrast-induced nephropathy in mice. PLoS One. 10:e0144329.

Djahanguiri B. 1969. The production of acute gastric ulceration by indomethacin in the rat. Scand J Gastroenterol. 4:265–267.

Farzaei MH, Abdollahi M, Rahimi R. 2015. Role of dietary polyphenols in the management of peptic ulcer. World J Gastroenterol. 21:6517–6522.

Goldstein JL, Swain T. 1965. The inhibition of enzymes by tannins. Phytochemistry. 4:185–192.

Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y. 2005. Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine. 12:656–662.

Hiruma-Lima CA, Batista LM, Almeida ABA, Magri LP, Santos LC, Vilegas W, Brito ALM. 2009. Antiulcerogenic action of ethanolic extract of the resin from Virola surinamensis Warb. (Myristicaceae). J Ethnopharmacol. 122:406–409.

Kahraman A, Erkasap N, Koken T, Serteser M, Aktepe F, Erkasap S. 2003. The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. Toxicology. 183:133–142.

Kim S, Jun C, Suk K, Choi B, Lim H, Park S, Lee SH, Shin H, Kim D, Shin T. 2006. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. Toxicol Sci. 91:123–131.

Krepsky PB, Isidório RG, Souza Filho JD, Côrtes SF, Braga FC. 2012. Chemical composition and vasodilatation induced by Cuphea carthagenensis preparations. Phytomedicine. 19:953–957.

Laine L, Takeuchi K, Tarnawski A. 2008. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology. 135:41–60.

Lee JK. 2011. Anti-inflammatory effects of eriodictyol in lipopolysaccharide-stimulated raw 264.7 murine macrophages. Arch Pharm Res. 34:671–679.

Lobô LT, Castro KCF, Arruda MSP, Silva MN, Arruda AC, Müller AH, Arruda GMSp, Santos AS, Souza Filho APS. 2008. Potencial alelopático de catequinas de Tachigali myrmecophylla (Leguminosae). Quim Nova. 31:493–497.

Monteforte MA, Lanuza F, Mondello F, Naccari C, Pergolizzi S, Galati EM. 2014. Phytochemical composition and gastroprotective effect of Feijoa sellowiana Berg fruits from Sicily. J Cost Life Med. 2:14–21.

Mota KS, de L, Dias GEN, Pinto MEF, Luiz-Ferreira A, Souza-Brito ARM, Hiruma-Lima CA, Barbosa-Filho JM, Batista LM. 2009. Flavonoids with gastroprotective activity. Molecules. 14:979–1012.

OECD. 2001. Test No. 423. In: OECD guidelines for testing of chemical organization for economic cooperation and development. Paris, France; p. 1–14.

Olate VR, Pertino MW, Theoduloz C, Yesilada E, Monsalve F, González P, Droguedt D, Richomme P, Hadi AHA. 2012. New gastroprotective labdaneamides from (4S,9R,10R) methyl 18-carboxy-labda-8,13(E)-diene-15-oate. Planta Med. 78:362–367.

Oyagi A, Ogawa K, Kakino M, Hara H. 2010. Protective effects of a gastrointestinal agent containing Korean red ginseng on gastric ulcer models in mice. BMC Complement Altern Med. 10:45.

Pal C, Bindu D, Suy D, Alam A, Goyal M, Iqbal MS, Maity P, Adhikari SS, Bandyopadhyay U. 2010. Gallic acid prevents nonsteroidal anti-inflammatory drug-induced gastropathy in rat by blocking oxidative stress and apoptosis. Free Radic Biol Med. 49:258–267.

Pezet R, Perret C, Tabacchi R. 2001. Analysis of oligomeric and polymeric tannins of grape berries by liquid chromatography/electrospray ionization multiple-stage tandem mass spectrometry. Eur J Mass Spectrom. 7:419–426.

Re R, Pellegrini N, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med. 26:1231–1237.

Rios LY, Bennett RN, Lazarus AS, Rémesy C, Scalbert A, Williamson G. 2002. Cocoa procyanidins are stable during gastric transit in humans. Am J Clin Nutr. 76:1106–1110.

Rodrigues WA. 1980. Revisão taxonômica das species (voucher number) de Virola Aublet. (Myristicaceae) do Brasil. Acta Amaz. 11:127.

Sun J, Shi J, Jiang Y, Xue SI, Wei X. 2007. Identification of two polyphenolic compounds with antioxidant activities in Longan pericarp tissues. J Agric Food Chem. 55:5864–5868.

Szelenyi I, Thiemer K. 1978. Distention ulcer as a model for testing of drugs with gastroprotective action. Arch Toxicol. 41:99–105.

Thirunavukkarasu P, Ramkumar L, Ramanathan T. 2009. Anti-ulcer activity of Exocarica agallocha bark on NSAID-induced gastric ulcer in albino rats. Global J Pharmacol. 3:123–126.

Yilmaz Y, Toledo RT. 2004. Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. J Agric Food Chem. 52:255–260.