

# Gastroprotective activity of the hydroethanolic extract and ethyl acetate fraction from *Kalanchoe pinnata* (Lam.) Pers.

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Peptic ulcers are an important pathology, and the search for safer and more effective treatment methods is of paramount importance. In this study, we assess the gastroprotective effects of the hydroethanolic extract (HE) and ethyl acetate fraction (EAF) from *Kalanchoe pinnata* leaves against an ethanol/HCl-induced ulcer model in rats. The HE reduced gastric lesions by approximately 47% (400 mg/kg). A significant inhibition of the gastric lesions by 50% was observed after pretreatment with the EAF (200 mg/kg). Quercetrin and quercetin 3-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside were isolated and identified in the flavonoid fraction (EAF) by HPLC and NMR analyses because this fraction showed the highest gastroprotective effect. This fraction demonstrated high antioxidant activities (CE<sub>50</sub>=41.91  $\mu$ g/mL) by DPPH in comparison with Trolox<sup>®</sup> and 11.33 mmol Trolox<sup>®</sup> equivalent by ORAC. In conclusion, the HE and EAF from *K. pinnata* displayed gastroprotective activity in rats, most likely due to the presence of flavonoids.

**Uniterms:** Gastric ulcer/study/rats. Gastric ulcer/treatment. *Kalanchoe pinnata*/chromatographic profile. *Kalanchoe pinnata*/gastroprotective activity. Quercetrin. Quercetin 3-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside.

## INTRODUCTION

For the past two centuries, peptic ulcers have caused high morbidity and mortality (Malfertheiner, Chan, Mccoll, 2009). This disease is a serious and prevalent pathology (Calam, Baron, 2001). A peptic ulcer is a chronic lesion, usually single, that can be located in the gastric or duodenal mucosae (Kumar, Abbas, Fausto, 2005). In the UK, the incidence of this disease was 12% higher in men than in women (Araujo, Borini, Guimarães, 2014). A review article (January 1980 to February 2009) showed that the incidence rate of uncomplicated peptic ulcers was approximately one case per 1000 people per year in the general population; the incidence rate for complicated peptic ulcers that included bleeding and perforation was approximately 0.7 cases per 1000 people per year (Lin, Rodríguez, Hernández-Díaz, 2011).

The pathophysiology of peptic ulcers suggests that this disease is caused by an imbalance between defensive factors (mucus and bicarbonate secretion, mucosal barrier, blood flow, and prostaglandins) and destructive factors (reactive oxygen species and acid and pepsin secretion), resulting in the acute inflammation of the gastrointestinal mucosae (Laine, Takeuchi, Tarnawski, 2008). Several stimuli serve as offensive factors, including *Helicobacter pylori*, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), stress, alcohol and cigarette smoking (Malfertheiner, Chan, Mccoll, 2009; Malnick *et al.*, 2014). The current medical treatment for peptic ulcers is generally based on proton pump inhibitors and histamine H<sub>2</sub>-receptor blockers (Jain *et al.*, 2007). However, the long-term use of these therapeutic agents can produce adverse effects, such as hypergastrinemia and hyperplasia in the enterochromaffin-like (ECL) cells, the risk of pneumonia, diarrhea and the disturbance of human gastrointestinal microflora (Orlando, Lenard, Orlando, 2007; Bays, Finch, 1990; Devault, Talley, 2009; Ksiadzyna, Szelag, Paradowski, 2015). Therefore, the search for safer and more effective alternatives to treat

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gastric ulcers is of paramount importance. Medicinal plants are considered an excellent alternative and have been used to cure ailments for centuries (Zhang *et al.*, 2015). Many studies have reported that medicinal plants and the compounds isolated from them, such as flavonoids, have gastroprotective activities (Awaad, El-Meligy, Soliman, 2013). Unfortunately, there is a lack of complete information regarding the pharmacokinetic factors (absorption, distribution, metabolism and elimination) and pharmacodynamic factors of medicinal plants (Mancuso, 2015). Herbal medicines include many bioactive compounds that can modify drug disposition. One example is the possibility of interactions with drug metabolizing enzymes, such as isoforms of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) and sulfotransferases (SULT) or transporters (Brantley *et al.*, 2014), leading to the possibility of side-effects, mainly when co-administered with another medication. Polyphenolic compounds are often found in natural products. Their metabolism is mediated by (UGT) in the intestine and liver. The inhibition of UGTs in the intestine, which is the first barrier to oral xenobiotics, by these compounds could alter drug disposition (Roe *et al.*, 2016). Therefore, medical supervision is important because the overuse of herbal medicines can possibly pose health risks (Mancuso, 2015).

*Kalanchoe pinnata* (Lam.) Pers. (synonym: *Bryophyllum pinnatum*) (The Plant List, 2015) is a perennial herb. This species is popularly known in Brazil as “folha-da-fortuna,” “coirama,” “courama,” “courama-vermelha,” and “saião roxo”. In Brazil and other parts of the world, it is widely used to treat diarrhea and vomiting, earache, gastric ulcers, burns, abscesses, and insect bites (Almeida *et al.*, 2000; Afzal *et al.*, 2012). The leaves of this plant are reported to possess wound healing (Nayak, Marshall, Isitor, 2010), anti-inflammatory (Ferreira *et al.*, 2014), antioxidant (Hazarika *et al.*, 2012), antimicrobial (El Abdellaoui *et al.*, 2010) and antileishmanial properties (Muzitano *et al.*, 2006a). Pal, Chaudhuri (1991), Adesanwo *et al.*, (2007); Braz, Oliveira, Viana (2013) have reported the antiulcer activity of the methanolic fraction and the methanol and aqueous extract of *K. pinnata*. Phytochemical investigations have shown the presence of tannins, alkaloids and saponins (Biswas *et al.*, 2011), bufadienolides (Yamagishi *et al.*, 1989) and, mainly, flavonoids (Fürer *et al.*, 2013) in *K. pinnata*.

In this study, we demonstrate the gastroprotective effect of the hydroethanolic extract and the ethyl acetate fraction from the leaves of *Kalanchoe pinnata* against ethanol-acidified gastric ulcer in rats. In parallel with the pharmacological study, a chemical analysis was

performed; flavonoids were shown to be the main compounds in the fraction.

## MATERIAL AND METHODS

### Plant material

The leaves of *Kalanchoe pinnata* (Lam.) Pers. Crassulaceae were collected at the Chemical, Biological and Agricultural Pluridisciplinary Research Center-Campinas State University (CPQBA-UNICAMP), Paulínia, São Paulo, Brazil. A voucher specimen was deposited at Collection of Medicinal and Aromatic Plants (CQBA-UNICAMP) under the number 337.

### Extract preparation

The fresh leaves were cut into small pieces and dried at 40 °C. The ethanol:water (7:3) extract was prepared by maceration for 7 days. The extract was concentrated under reduced pressure and then lyophilized. The hydroethanolic extract was partitioned using chloroform, ethyl acetate and water.

### HPLC analysis and identification of the compounds

Briefly, the separation of the ethyl acetate fraction was performed in a Shimadzu® model LC-20 AT instrument with a diode array detector (DAD). The separation was carried out on a 250 mm x 4.6 mm, 5 µm Shim-pack VP-ODS C18 reverse-phase column (Shimadzu®). HPLC-grade acetonitrile (solvent B) and purified water with 0.1% trifluoroacetic acid (solvent A) were used as the mobile phase. The gradient program used was as follows: (1) 5% solvent B for 0-5 min and then (2) 5-100% solvent B for 60 min. The flow rate was 1 mL/min, 20 µL samples (1 mg/mL) were injected, and the detection occurred at 254 nm. The isolation of the major constituents was achieved on an HPLC semi-preparative UV system (Shimadzu® - C18 Shim-pack PREP-ODS 250 mm x 20 mm, 5 µm, flow rate 10 mL/min). The mobile phase consisted of 25% acetonitrile and water with 0.1% trifluoroacetic acid, and the detection occurred at 254 nm. The major compounds were identified by <sup>1</sup>H and <sup>13</sup>C NMR spectra on a Bruker Avance DRX-300 (<sup>1</sup>H: 300 MHz; <sup>13</sup>C: 75 MHz) using CD<sub>3</sub>OD as the solvent.

### Total flavonoid content

Flavonoids were quantified using the adapted

methodology of Noriega *et al.* (2012). Twenty microliters of hydroethanolic extract and ethyl acetate fraction from the *K. pinnata* leaves in 60% ethanol were added to 20  $\mu\text{L}$  of 2%  $\text{AlCl}_3$  in a 96-well microplate. A sixty percent ethanol solution was added to bring the volume to 250  $\mu\text{L}$ . Quercetin was used as a standard for the calibration curve. The absorbance was measured after 30 min at 430 nm in a Synergy™ HT Multi-Detection Microplate Reader. All the analyses were performed in triplicate, and the results were expressed as a quercetin equivalent (mg of quercetin/g of dry weight of test sample).

### Total phenolic content

The total phenolic content was determined using the Folin Ciocalteu method adapted from Ainsworth; Gillepsie (2007). In a 96-well microplate, 20  $\mu\text{L}$  of the hydroethanolic extract, ethyl acetate and aqueous fractions from the *K. pinnata* leaves were mixed with 50  $\mu\text{L}$  of Folin-Ciocalteu reagent and 100  $\mu\text{L}$  of saturated sodium carbonate (700 mM). After two hours, in the absence of light, the absorbance was measured at 765 nm using a Synergy™ HT Multi-Detection Microplate Reader. Gallic acid was used for the calibration curve. All the analyses were performed in triplicate, and the results were expressed as gallic equivalent (mg of gallic acid/g of dry weight of test sample).

### Antioxidant activity

The scavenging activity of the hydroethanolic extract and ethyl acetate fraction was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Brand-Williams, Cuvelier, Berset, 1995). Briefly, 50  $\mu\text{L}$  of the samples, Trolox® (standard compound) at varying concentrations and 150  $\mu\text{L}$  of DPPH (175  $\mu\text{M}$ ) were added to a 96-well microplate. The samples, Trolox® and DPPH were solubilized in 80% methanol. A control solution, without a test sample, was prepared with DPPH solution and 80% methanol. All the analyses were completed in triplicate. After 2 hours of reaction with absence of light, the absorbance was measured in a Synergy™ HT Multi-Detection Microplate Reader at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH: Scavenging effect (\%)} = [(Abs_c - Abs_s) / Abs_c] \times 100$$

where  $Abs_c$  is the absorbance of the control reaction, and  $Abs_s$  is the absorbance of the sample or Trolox®.

The  $Ec_{50}$  value was calculated as the concentration of the sample required to scavenge the DPPH radical by 50%.

The ORAC (Oxygen Radical Absorbance Capacity) assay was carried out using an automated microplate reader. Filters were selected for an excitation wavelength of 485 nm and an emission wavelength of 515 nm. In addition, AAPH was used as a peroxy radical generator, Trolox® as a standard, and fluorescein as a fluorescent probe. In 96-well microtiter plates, twenty-five microliters of hydroethanolic extract (10 mg/mL), ethyl acetate fraction (10 mg/mL) from *K. pinnata*, blank or Trolox® calibration solution (6.25 to 100  $\mu\text{M}$ ) were mixed with 150  $\mu\text{L}$  of fluorescein (40 nM) and incubated for 15 min at 37 °C before injecting 25  $\mu\text{L}$  of the AAPH solution (173 mM). The fluorescence was measured every minute for 1 h. The final ORAC values were calculated using the net area under the decay curves. The results were expressed as mmol of Trolox® equivalent (TE)/g of dry weight of the test sample (Prior *et al.*, 2003).

### In vivo assays

#### Animals

Male Wistar rats (150 to 180 g) from the Central Animal House of the School of Pharmaceutical Sciences and the Chemistry Institute of the University of São Paulo were used. The animals were kept under the following standard conditions: (1) food and water *ad libitum*, (2) 12 h dark-12 h light, (3) temperature of  $22 \pm 2$  °C, and (4)  $55 \pm 10\%$  humidity. The samples (hydroethanolic extract and ethyl acetate fraction), cloprostenol (standard drug) and water (used as the vehicle) were administered by gavage. The Commission of Ethics in Animal Experimentation of FCF-USP approved all the procedures under number 426.

#### Ethanol/HCl-induced ulcer model

The procedure was performed according to the method described by Mizui; Doteuchi (1983). The rats were fasted for 12 hours and were kept in cages with raised floors of wide mesh to prevent coprophagy. The animals were divided into the following five treatment groups: (1) vehicle (water 10 mL/kg b.w.), (2) cloprostenol (150  $\mu\text{g}/\text{kg}$ ) (Neugebauer *et al.*, 1992), (3) hydroethanolic extract (100, 200 and 400 mg/kg) and (4) ethyl acetate fraction (50, 100 and 200 mg/kg). Following 30 minutes of oral treatment with the above samples, the gastric lesions were induced with 300 mmol/L HCl in 60% ethanol (10 mL/kg). One hour later, the animals were sacrificed, and the stomachs were removed and opened along the greater curvature. The injuries were analyzed using Image Pro-Plus® software, and a relative lesion area was determined (RLA in %). The relative lesion area was determined as follows:

RLA = (TLA x 100)/TA, where TLA= total lesion area (mm<sup>2</sup>), and TA= total stomach area (mm<sup>2</sup>).

### Statistical analysis

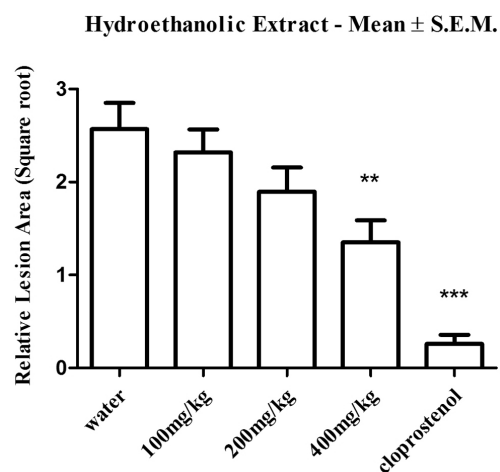
The values were transformed using the square root method to normalize the data distribution. The results were expressed as the mean ± S.E.M. and analyzed by one-way ANOVA followed by Dunnett's post-hoc test using GraphPad Prism 5 software. The results were considered statistically significant when the *p*-value was less than 0.05.

### RESULTS/DISCUSSION

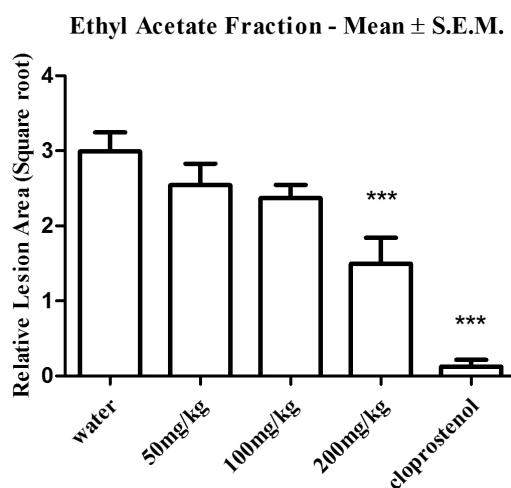
The ethanol/HCl-induced ulcer model is a well-established method for the evaluation of gastroprotective effects. Ethanol produces an inflammatory response, consequently destroying the stomach-protecting layers and provoking hemorrhagic ulceration of the stomach in experimental animals (Gazzieri *et al.*, 2007; Castro *et al.*, 2010). Moreover, ethanol in contact with gastric mucosa produces oxygen free radicals (Pan *et al.*, 2008). The role of HCl in the ethanol solution is to accelerate gastric ulcer formation (Mizui, Doteuchi, 1983). The gastroprotective effects of the hydroethanolic extract and ethyl acetate fraction from the leaves of *Kalanchoe pinnata* were assessed using this model.

The results obtained from the ethanol/HCl model showed that the hydroethanolic extract exerted 47% (*p*<0.01) protection on the gastric mucosa at a dose of 400 mg/kg (Figure 1). Pretreatment with the ethyl acetate fraction at 200 mg/kg dose significantly inhibited (*p*<0.001) gastric ulcers by 50% (Figure 2). For both experiments, cloprostamol, an analogue of PGF<sub>2</sub> alpha, was used as a positive control because it is related to mucosal defense (Neugebauer *et al.*, 1992). Prostaglandins can inhibit gastric acid secretion and stimulate mucus and bicarbonate secretion and mucosal blood flow to promote ulcer healing (Wallace, 2008). A previous study with methanolic extract (20 mg/kg and 40 mg/kg) in an ulcer model induced by indomethacin demonstrated inhibition indices of 49.39% and 71.08%, respectively (Adesanwo *et al.*, 2007). In contrast to this study, other studies using an aqueous extract (1 g/kg and 2 g/kg) in an indomethacin model and a methanolic fraction (100 mg/kg and 300 mg/kg) in an ulcer-induction ethanol model of leaves from *K. pinnata* showed the inhibition of gastric lesions by 45.49% and 49.50% and 37.5% and 54.2%, respectively (Pal, Chaudhuri, 1991; Braz, Oliveira, Viana 2013). However, our results showed that the ethyl acetate fraction displayed superior antiulcer activity by 50% at a dose of

200 mg/kg. Non-coincident results by different authors can be explained by the use of different extraction solvents. Studies reported that different methods of extraction and solvent systems used to extract bioactive compounds from medicinal plants are an important issue to consider because these factors have an influence on biological activities (Hayouni *et al.*, 2007; Murugan, Parimelazhagan, 2014).



**FIGURE 1** - Effect of the hydroethanolic extract from *Kalanchoe pinnata* leaves on the ethanol/HCl-induced gastric model in Wistar rats. Relative lesion area is presented as the mean ± S.E.M.; n=7 for each group. ANOVA was carried out and followed by Dunnett's test, \*\* *p* < 0.01; \*\*\* *p* < 0.001 compared to water.



**FIGURE 2** - Effect of the ethyl acetate fraction from *Kalanchoe pinnata* leaves on the ethanol/HCl-induced gastric model in Wistar rats. Relative lesion area is presented as the mean ± S.E.M.; n=7 for each group. ANOVA was carried out and followed by Dunnett's test, \*\*\* *p* < 0.001 compared to water.

Chemical analyses were performed with the aim of isolating and identifying the major compounds in the ethyl acetate fraction because this sample showed the

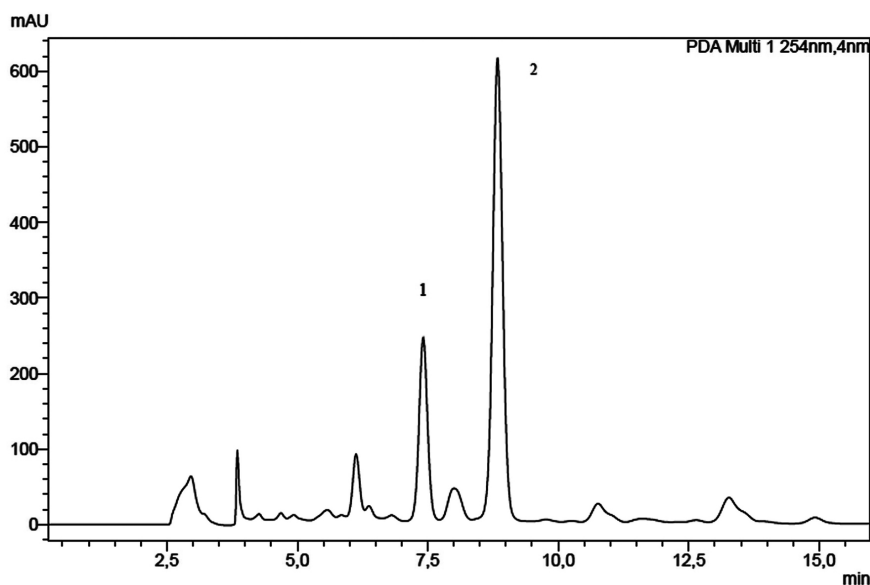
best results in the antiulcer assay. This study, however, is the first to establish a relationship between the major compounds and their activity. Many studies have shown that flavonoids interact with gastric ulcers. Jung *et al.*, (2007) reported that the flavonoid-rich fraction from *Orostachys japonicus* (Crassulaceae) reduced HCl/ethanol-induced gastric lesions and that the anti-ulcer activity could be attributed to the flavonoid compounds. Batista *et al.*, (2013) demonstrated that the flavonoid-rich fraction from *Syngonanthus macrolepis* (Eriocaulaceae) at a dose of 100 mg/kg protected the gastric mucosa in animals, most likely due to the involvement of sulfhydryl compounds and the antioxidant system.

In our study, the HPLC-DAD analysis (Figure 3) of the ethyl acetate fraction indicated two major compounds (1 and 2). These substances were isolated and identified by  $^1\text{H}$  and  $^{13}\text{C}$  NMR (using  $\text{CD}_3\text{OD}$  as the solvent).

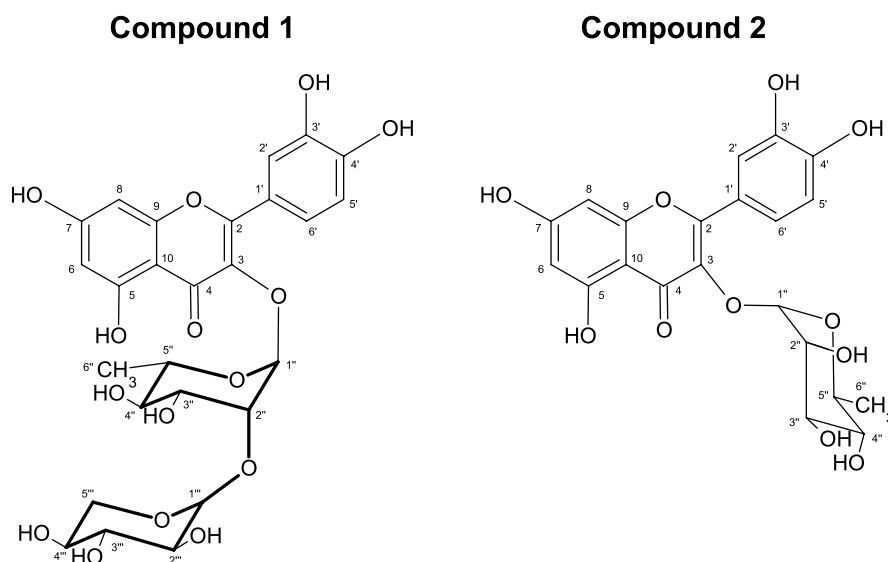
Compound 1 (Figure 4) was identified as quercetin 3-*O*- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside by  $^1\text{H}$  NMR (300 MHz) and  $^{13}\text{C}$  NMR (75 MHz).  $^1\text{H}$ -NMR ( $\text{CD}_3\text{OD}$ , 300 MHz) spectra showed peaks at  $\delta$  7.37 (d,  $J = 1.8$  Hz, H-2'), 7.00 (dd,  $J = 8.4, 2.1$  Hz, H-6'), 6.93 (d,  $J = 8.4$  Hz, H-5'), 6.37 (d,  $J = 2.1$  Hz, H-8), 6.21 (d,  $J = 2.1$  Hz, H-6), 5.38 (d,  $J = 0.9$  Hz, H-1''), 4.21 (d,  $J = 7.2$  Hz, H-1'''), 4.19 (m, H-2''), 3.9 (m, H-3''), 3.8 (m, H-5''), 3.74 (m, H-4'''), 3.66 (dd,  $J = 12.9, 2.0$  Hz, H-5'''), 3.56 (dd,  $J = 9.3, 7.2$  Hz, H-2'''), 3.47 (dd,  $J = 9.3, 3$  Hz, H-3'''), 3.39 (d,  $J = 11.4$  Hz, H-5'''), 3.35 (dd,  $J = 9.3, 9.3$  Hz, H-4''), and 1.02 (d,  $J = 6.3$  Hz, H-6'').  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ , 75 MHz) showed peaks at  $\delta$  179.85 (C-4), 165.86 (C-7), 163.25

(C-9), 159.21 (C-5), 158.55 (C-2), 149.86 (C-4'), 146.52 (C-3'), 136.88 (C-3), 122.99 (C-1'), 122.77 (C-6'), 116.91 (C-2'), 116.55 (C-5'), 107.83 (C-1'''), 105.91 (C-10), 103.28 (C-1''), 99.88 (C-6), 94.81 (C-8), 82.72 (C-2''), 74.37 (C-4''), 73.84 (C-3'''), 72.86 (C-2'''), 72.06 (C-5''), 71.82 (C-3''), 69.90 (C-4'''), 67.47 (C-5'''), and 17.76 (C-6''). Our results were compared and were consistent with the results presented by Muzitano *et al.* (2006a), who also reported that quercetin 3-*O*- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside may be a chemical marker for *K. pinnata* because it is not common in other plant species, except for *Alphitonia philippinensis* (Rhamnaceae) and *Rollinia emarginata* (Annonaceae) (Jou *et al.*, 2004; Roth *et al.*, 2011).

Compound 2 (Figure 4) was identified as quercitrin. Our results were compared and found to be consistent with those described by Hanamura; Hagiwara; Kawagishi (2005) [47].  $^1\text{H}$ -NMR ( $\text{CD}_3\text{OD}$ , 300 MHz) spectra showed peaks at  $\delta$  7.35 (d,  $J = 1.7$  Hz, H-2'), 7.30 (dd,  $J = 8.3, 1.8$  Hz, H-6'), 6.92 (d,  $J = 8.3$  Hz, H-5'), 6.35 (d,  $J = 1.7$  Hz, H-8), 6.19 (d,  $J = 1.5$  Hz, H-6), 5.36 (s, H-1''), 4.25 (s br, H-2''), 3.78 (dd,  $J = 9, 3.2$  Hz, H-3''), 3.43 (dd,  $J = 9.6, 6$  Hz, H-5''), 3.35 (t,  $J = 9$  Hz, H-4''), and 0.95 (d,  $J = 6.4$  Hz, H-6'').  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ , 75 MHz) showed peaks at  $\delta$  179.68 (C-4), 165.82 (C-7), 163.18 (C-5), 159.37 (C-2), 158.52 (C-9), 149.79 (C-4'), 146.41 (C-4'), 136.32 (C-3), 123.10 (C-6'), 123.05 (C-1'), 117.13 (C-5'), 116.51 (C-2'), 103.58 (C-1''), 99.95 (C-6), 94.88 (C-8), 73.39 (C-4''), 72.24 (C-2''), 72.10 (C-3''), 71.99 (C-5''), and 17.77 (C-6'').



**FIGURE 3** - HPLC-DAD ( $\lambda=254$  nm) separation of the ethyl acetate fraction from *Kalanchoe pinnata* leaves. The separation was carried out on a Shimadzu LC-20A series, 250 mm x 4.6 mm, 5  $\mu\text{m}$  Shim-pack VP-ODS C18 reverse-phase column (Shimadzu<sup>®</sup>), at flow rate of 1 mL/min. Isocratic elution (25% acetonitrile and water with 0.1% trifluoroacetic acid) in 15 min.



**FIGURE 4** - Structural formulas of compound 1 quercetin 3-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside and compound 2 quercitrin isolated from the *Kalanchoe pinnata* leaves.

The isolated compounds are flavonoids. Flavonoids are phenolic substances with many health properties that frequently have antioxidant activities as well (Benavente-García *et al.*, 1997). Previous studies have shown that *K. pinnata* is an important source of phenolic compounds, mainly flavonoids (Costa *et al.*, 2008). Many flavonoids have been previously described in this species, including the compounds isolated in this study (Muzitano *et al.*, 2006a, 2006b; Tatsimo *et al.*, 2012). The literature reports that compound 1 (diglycosylated flavonoid) showed antiallergic actions (Ichikawa, Ogura, Lijima, 1986), high antioxidant potential (Nascimento *et al.*, 2013) and antileishmanial activities (Muzitano *et al.*, 2006a). Moreover, quercitrin (2) protected skin from UVB-induced inflammation (Yin *et al.*, 2013), displayed antioxidant (Aderogba, Okoh, Idowu, 2005; Tai *et al.*, 2014) and antileishmanial activities (Muzitano *et al.*, 2006b), showed antiproliferative and apoptotic effects in colon cancer cells (Cincin *et al.*, 2015), demonstrated gastroprotective actions (Mota *et al.*, 2009) and showed intestinal anti-inflammatory effects (Camuesco *et al.*, 2004). Júnior *et al.*, (2014) reported that quercitrin was present in *Croton campestris* extracts at 2.34% and might be involved in gastroprotective activities against ethanol, acidified ethanol-, and indomethacin-induced gastric lesions. This antiulcer effect seems to be mediated by NO and endogenous prostaglandin pathways. Quercetin is a structure common to both isolated compounds in our lab. This molecule could be responsible for antiulcer activity. Kahraman *et al.*, (2003) found that the treatment of rats with quercetin (200 mg/kg) inhibited ethanol-induced

gastric lesions and suggested that antiperoxidative and antihistaminic effects and antioxidant mechanisms mediated this effect. Quercetin was also related to gastroprotection in indomethacin-induced gastric damage (Shakeerabanu *et al.*, 2011).

In our experiments, the total amount of flavonoid and phenolic compounds in the ethyl acetate fraction was higher than that in the hydroethanolic extract and aqueous fractions (Table I). Because many studies revealed a relationship between phenolic compounds and antioxidant activity (Popa *et al.*, 2012; Jemia *et al.*, 2013), the antioxidant activity was also assessed to determine the possible mechanisms of action.

The antioxidant activity of flavonoids plays an important role in gastroprotective activities (Alvarez-Suarez *et al.*, 2011). Studies have demonstrated that these compounds can scavenge superoxide, hydroxyl and peroxy radicals. Moreover, they are involved in lipid peroxidation inhibition, mucus production, decrease of histamine levels and inhibition of gastric acid secretion (Priya, Parminder, Jaspreet, 2012; Repetto, Llesuy, 2002). Li *et al.*, (2015) reported that the flavonoids from *Abrus cantoniensis* extracted with ethanol had an antiulcer effect, probably due to their antioxidant activity.

There are two methods of measuring total antioxidant capacity: assays based on hydrogen atom transfer (HAT), such as ORAC, and assays based on electron transfer (ET), such as DPPH. It is advisable to use two or more different methods of antioxidant capacity measurement because of the different mechanisms of action of antioxidants (Huang, Ou, Prior, 2005). The results of the antioxidant capacity

**TABLE I** - Total flavonoid and phenolic compound content in the hydroethanolic extract and the ethyl acetate fraction from *Kalanchoe pinnata* leaves. The data shown as the mean  $\pm$  S.E.M. ; n=3.

Sample	Total Flavonoid Content <sup>a</sup>	Total Phenolic Content <sup>b</sup>
Hydroethanolic extract	36.9 $\pm$ 4.0	149.6 $\pm$ 2.2
Ethyl acetate fraction	164.3 $\pm$ 5.4	363.3 $\pm$ 6.4

<sup>a</sup>Total flavonoid content is expressed as the mean  $\pm$  S.E.M. mg of quercetin/g of dry weight. <sup>b</sup>Total phenolic content is expressed as the mean  $\pm$  S.E.M. mg of gallic acid/g of dry weight.

experiments by DPPH showed that the ethyl acetate fraction ( $CE_{50} = 41.91 \mu\text{g/mL}$ ) was more active than the hydroethanolic extract ( $CE_{50} = 72.63 \mu\text{g/mL}$ ). The  $CE_{50}$  of Trolox<sup>®</sup>, the reference compound, was ( $CE_{50} = 33.38 \mu\text{g/mL}$ ). However, Tatismo *et al.*, (2012) found that the antioxidant activity of the MeOH extract of *K. pinnata* ( $CE_{50} = 52.48 \mu\text{g/mL}$ ) was higher than that of the ethyl acetate fraction ( $CE_{50} = 78.11 \mu\text{g/mL}$ ). The values obtained by the ORAC method for the hydroethanolic extract and the ethyl acetate fraction were 4.31 mmol TE/g and 11.33 mmol TE/g, respectively. The antioxidant capacity was higher in the ethyl acetate fraction in both assays (DPPH and ORAC).

These data suggest that the gastroprotective effect is due, at least partially, to flavonoids and the antioxidant capacity of these substances in all samples tested.

## CONCLUSION

Our results provide evidence for the popular use of *Kalanchoe pinnata* in treating gastric ulcer. This can be confirmed by the gastroprotective potential of *K. pinnata* in the animal model. The ethyl acetate fraction reduced gastric lesions by 50% at a dose of 200 mg/kg, most likely due to the high amounts of phenolic and flavonoid compounds present in this fraction. Studies are currently being conducted by our group to investigate the mechanisms of action of the flavonoid fraction present in this species.

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