Inclusion of vitexin in β-cyclodextrin: preparation, characterization and expectorant/antitussive activities

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ABSTRACT

The study aimed to include the isolated vitexin of Jatropha mutabilis in the β-cyclodextrin cavity to improve the solubility of this flavone. Its characterization was performed by techniques such as 1H NMR/ROESY (Nuclear Magnetic Resonance Spectroscopy), FT-IR (Infrared Spectroscopy with Fourier Transform), SEM (Morphological analysis of IC by Scanning Electron Microscopy) and dissolution study in vitro. In addition, the following activities were evaluated in the animal models: expectorant, phenol red dosage in bronchoalveolar lavage and antitussive, cough induced by citric acid. In the characterization of the complex, interaction between hydrogens of ring B of vitexin and (H3) of β-CD was observed, in addition to changes in morphology. In the dissolution test, an increase in the rate of dissolution of vitexin was observed in the first 30 min for the CI vitexin/β-CD when compared with vitexin. Regarding the pharmacological activity, it was observed that the inclusion complex (IC) vitexin/β-CD presented higher expectorant activity when compared to vitexin (p < 0.05), suggesting increased bioavailability. As for the antitussive activity, both vitexin and the complex had similar effects and were dose independent. In the toxicity test using Artemia salina, vitexin and IC vitexin/β-CD were considered non-toxic. At last, the study efficacy of vitexin/β-CD IC as an expectorant and of vitexin as antitussive. All of these data are being described for the first time.

1. Introduction

Vitexin is a C-glycosylated flavone, being chemically known as 8-D-glucosyl-4',5,7-trihydroxy-flavone or apigenin-8-C-glucosidic. It has several pharmacological activities in vivo and in vitro, particularly anticancer, antioxidant, anti-inflammatory, antinociceptive, antihypertensive, antispasmodic, antiviral and antidepressant [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Researches have showed that this flavone (400 μg/kg) reduces the influx of pulmonary neutrophils by 80% in mice exposed to lipopolysaccharide aerosols [13]. Recently, it has been demonstrated that vitexin (0.2, 1 and 5 mg/kg) inhibits inflammation in allergic asthma induced by ovalbumin [14]. However, a study of the metabolism of vitexin in rats showed that it is poorly absorbed in the gastrointestinal tract [15] and has low bioavailability [16, 17]. Once that is probably due to the low solubility in water (7.62 μg/mL), it is important to develop methodologies improving the solubility of vitexin in aqueous medium so that its therapeutic action is improved.

In this context, a group of molecules classified as cyclic oligosaccharides is being used in the development of pharmaceuticals, particularly due to their complexing properties, promoting an increase in the solubility of few soluble drugs. These oligosaccharides are called cyclodextrins (CDs) [18]. CDs encapsulate molecules/drugs, acting as a vehicle and improving their bioavailability. Parental or first-generation CDs are of three types (α-CD, β-CD and γ-CD) and have, respectively, six, seven and eight α-(1,4)-glycosyl [19]. Among them, β-CD is the most accessible because of the low cost and low toxicity [20]. The use of the various macrocyclic nanocarriers has been of great interest because it improves some physicochemical properties of substances, increasing the...
effectiveness of the treatment and reducing toxic or collateral effects [21]. Thus, inclusion complexes are used in the pharmaceutical field to increase the solubility, stability and bioavailability of drugs [22].

Few soluble drugs often exhibit lower in vivo bioavailability behavior when compared to those of high solubility. Therefore, the formation of complexes is among the most advantageous and efficient alternatives when seeking improvement in dissolution and bioavailability properties of poorly hydrophilic drugs [23, 24].

In light of the pharmacological potential of vitexin as an anti-inflammatory (mainly in the respiratory system, as previously cited) [13, 14], the low aqueous solubility and consequently the low availability, the present study aims to prepare and characterize the vitexin/β-CD inclusion complex with the aim of improving its solubility. The resulting complex will be characterized by techniques such as Nuclear Magnetic Resonance (NMR), SEM (Scanning Electron Microscopy), Fourier Transform Infrared (FT-IR) and dissolution study in vitro. In addition, we aim at further evaluating the in vivo expectorant and antitussive effects of both vitexin and the vitexin/β-CD inclusion complex obtained.

2. Material and methods

2.1. Material

Vitexin was isolated from Jatropha mutabilis (Pohl) Baill (Euphorbiaceae), (SISGEN code AB71SE4), a species collected in the Caatinga of the semi-arid region of Northeastern Brazil, Petrolina-PE, geographic coordinates 09°19’32.60″S, 40°32’51.20″W, 387 m altitude. The species was deposited in the herbarium of the Federal University of the Valley of São Francisco under the registration number of exsicata 14316. β-CD (MM = 1134.98, purity ≥98%) was obtained from ISP Technologies. All reagents used were of analytical grade.

2.2. Isolation of vitexin from Jatropha mutabilis

2.2.1. Isolation and identification

The leaves collected were submitted to drying in an air circulation drying oven at 40 °C for 8 days. The material (1.52 kg) was pulverized in a knife mill and macerated (25 °C) with 95% ethyl alcohol. Three extractions were performed in a 72-hour interval. The extractive solution was concentrated in a rotary evaporator (50 °C), yielding 56.5 g of the crude ethanolic extract (Jm-EEB). Jm-EEB (51.5 g) was solubilized in a mixture of methanol and water, MeOH:H2O (3:7, v/v), under mechanical agitation for 40 min, resulting in the formation of the hydroalcoholic solution. This solution was subjected to the sequenced liquid/liquid partition in the separatory funnel with the following solvents in the order of increasing polarity, hexane (Jm-H, 14.59 g), chloroform (Jm-CI, 1.82 g) and ethyl acetate (Jm-AcOEt, 3.93 g). During the concentration of the AcOEt phase in the evaporator route, a yellow precipitate formed. This was purified by recrystallization with methanol to yield vitexin (Jm-1, 725.5 mg).

2.3. Preparation of the complex

Vitexin/β-CD IC were prepared in which both vitexin and β-CD (1:1 M), which was added to the methanol (~700 mL) sonicated in an ultrasonic apparatus for 15 min at 25 °C and then rotated (40 °C) for 40 min. The precipitate obtained was solubilized in water, filtered and subjected to drying in an air circulation oven at 40 °C. The precipitate obtained was solubilized in water, filtered for retention of non-complexed precipitate (vitexin, 9.9 mg) and submitted to drying with air circulation at 40 °C. After drying, the vitexin/β-CD complex (171.1 mg) was reserved. All calculations using the resulting complex were corrected to the vitexin equivalent retained on the filter paper, including for administration of the same to the animals, said difference was used for correction.

2.4. Characterization of the inclusion complex (IC)

2.4.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

1H-NMR (400 MHz), 13C NMR (125 MHz) and two-dimensional (COSY, HSQC, HMBD and ROESY) spectra of the samples were obtained with a Bruker Ascend™ 400 spectrometer. Vitexin was solubilized in DMSO (DMSO-d6). β-CD and vitexin/β-CD IC were solubilized in deuterated water (D2O).

2.4.2. Infrared Spectroscopy with Fourier Transform (FT-IR)

The FT-IR spectra were recorded on a Spectrum Two™ spectrometer from Perkin Elmer Version 10.4.00. The analysis was performed between 4000 and 400 cm⁻¹ and potassium bromide tablets were used for all samples.

2.4.3. Morphological analysis of IC by Scanning Electron Microscopy (SEM)

The sample was fixed in double-faced carbon adhesive and inserted in the stub with a 1.2-cm diameter and flat circular surface. Then, gold was deposited at a rate of 5 nm per minute for 5 min by the sputtering method using a QUORUM model Q15OR. The characterization through SEM was performed in a VEGA3 TESCAN microscope, operating with a voltage of 10 kV.

2.4.4. In vitro dissolution study

To determine the maximum absorption wavelength a vitexin solution was prepared with NaCl solution (0.15 M) acidified at pH 1.5 with HCl at 37 °C. Scanning was performed at wavelengths of 200–400 nm using an Even® UV-Vis spectrophotometer. Then a calibration curve at 340 nm (y = 0.0162x - 0.0216 and R² = 0.9957) was constructed by absorbances corresponding to the concentrations of 2, 10, 15, 20, 25 and 30 μg/ml vitexin in acidified NaCl (0.15 M) solution (pH 1.5) with HCl at 37 °C. To study the dissolution profile, 3 mg of free vitexin and the vitexin/β-CD complex (equivalence of 3 mg vitexin in the complex) were placed separately in 50 mL of acidified NaCl (0.15 M) solution (pH 1.5) with HCl. The experiment was performed under magnetic stirring (100 rpm) and temperature of 37 °C. Aliquots of the samples were collected over time and analyzed in a spectrophotometer at 340 nm to determine the amount of vitexin solubilized in the medium. All analyses were performed in triplicate [25, 26].

2.5. Expectorant and antitussive activities in mice

2.5.1. Animals

Male albino Swiss mice (Mus musculus), weighing between 25-30 g, were provided by the animal facility of the Universidade Federal do Vale do São Francisco (UNIVASF) (Petrolina-PE, Brazil). Prior to the experiments, all animals were kept in polypropylene cages at standard ambient conditions (22 ± 1 °C, 12/12 light/dark cycle) and with free access to feed and water. The animals fasted for 2 h prior to the experiment. All experimental procedures were approved by Animal Use Ethics Committee of the UNIVASF (CEUA-UNIVASF) under the number n° 0008/180816.

2.5.2. Expectorant activity

This assay was performed according [27]. Male mice weighing 25–30 g (n = 6/group) were pretreated orally with guaifenisin 100 mg/kg, vitexin suspended in distilled water (0.2, 1 or 5 mg/kg) [Venturini et al., 2018], vitexin/β-CD IC (dose of vitexin at 0.2, 1 or 5 mg/kg), β-CD (complexed vitexin vehicle) and distilled water (free vitexin vehicle). After 30 min, phenol red suspension (500 mg/kg) was administered intraperitoneally. After 30 min, the animals were anesthetized with sodium thiopental 160 mg/kg intraperitoneally and were euthanized. The bronchoalveolar lavage fluid (BALF) was obtained with 2 mL of saline (NaCl 0.9%), recovering approximately 1 mL of BALF, with 600 μL being centrifuged (2500 rpm) for 10 min. The supernatant (500 μL) was transferred to another vessel and 50 μL of NaOH 0.1 M was added. The
concentration of phenol red was measured by optical density at 565 nm wavelength and the results expressed in μg/mL. A standard curve (y = 0, 1741x + 0.0168 e R² = 0.99) was obtained with concentrations of 0.1–10 μg/mL of phenol red.

2.5.3. Antiinvasive activity

The cough reflex was induced as previously described [28, 29]. Briefly, mice were placed in a 500 mL glass chamber and individually exposed to a nebulized solution of citric acid 0.4 M for 3 min, and counted number of coughs produced before treatment. Mice (n = 6/group) with 4–19 coughs were randomly distributed and those who exhibited higher than 3 and lower than 20 coughing episodes were considered hyperresponsive and subresponsive, respectively, and were excluded from this assay. The coughing behavior was standardized by an abdominal contraction followed by a distinct opening of the mouth and how many times this happened during the exposure time. After 23 h and 30 min of the first exposure to citric acid, mice were treated orally with codeine (30 mg/kg), vitexin suspended in distilled water (0.2, 1 or 5 mg/kg), vitexin/β-CD IC (dose of vitexin at 0.2, 1 or 5 mg/kg in the complex), β-CD (vehicle of complexed vitexin) or distilled water (free vitexin vehicle) and, after 30 min, the animals were exposed to a nebulized solution of citric acid 0.4 M for 3 min, number of coughs produced were counted and compared to the previous exposure.

2.5.4. Statistical analysis

Numerical data were expressed as mean ± standard error of the mean (S.E.M.). The differences among the means were compared using the unpaired t test, one-way ANOVA followed to Dunnett’s post-test or Wilcoxon matched-pairs signed rank test using Graphpad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). The differences were considered significant when p < 0.05.

2.6. Toxicity test with Artemia salina

Toxicity was assessed using the lethality test methodology described by [30, 31]. Larvae of Artemia salina, a microcrustacean of the Anostraca class, were used as nauplii and the average lethal concentration (LC₅₀) was used as a parameter of biological activity.

A glass container (17 × 10 cm) filled with a solution of artificial sea salt (38 g/L) was used to hatch the eggs of Artemia salina. A plastic divider with several holes of approximately 2 mm was added to the vessel to form two unequal compartments. The eggs (25 mg) were placed in the largest compartment, protected from light, while the smallest compartment was illuminated. After 30 h, the A. salina larvae erupted and migrated to the lighted compartment.

Stock solutions of vitexin and complex were prepared at 5 mg/mL in water with 3% DMSO. Aliquots were added to 5 mL of sea salt solution and 10 larvae of A. salina were added. The referred aliquots were taken and solutions at concentrations of 1, 10, 100 and 1000 μg/mL were obtained. The test was performed in triplicate. A control was performed using a flask containing DMSO (3%) and sea salt solution. Ten larvae of the microcrustacean were also used.

3. Results and discussion

3.1. Structural elucidation of the isolated compound

After the unidimensional (Table 1) analyses of the NMR spectra (Figure 1) and comparison with the literature [32], it was possible to identify a common flavone in the genus as 8-C-β-D-glycosylapigenin or vitexin, described for the first time in Jatropha mutabilis.

Vitexin, yellow solid, 1H-NMR (500 MHz, DMSO-d₆): δ 4.7 (1H, d, J = 12 Hz, H-1'), 6.28 (1H, s, H-6), 6.8 (1H, s, H-3), 6.9 (2H, d, J = 8 Hz, H-3' and 5'), 8.0 (2H, d, J = 8 Hz, H-2' and 6'), 10.3 (1H, s, 4'-OH), 10.8 (1H, s, 7-OH), 13.17 (1H, s, 5-OH). 13C-NMR (125 MHz, DMSO-d₆): δ: 164.4 (C-2), 102.9 (C-3), 182.8 (C-4), 160.9 (C-5), 98.7 (C-6), 163.1 (C-7), 104.9 (C-8), 156.4 (C-9), 104.4 (C-10), 122.1 (C-1'), 129.7 (C-2' ≥ 6'), 116.8 (C-3', 5'), 161.5 (C-4'), 74.6 (C-1'), 71.4 (C-2'), 70.9 (C-3'), 71.09 (C-4'), 79.1 (C-5'), 61.8 (C-6').

Table 1. 1H-NMR, 13C (DMSO, 400 and 125 MHz), 1H-NMR x 13C - HSQC and HMBC data of vitexin.

| Hydrogen | 1H (ppm) (multi, J - Hz) | 13C - HSQC | 13C - HMBC |
|----------|---------------------------|-------------|-------------|
| OH       | 13.1 (s)                  |             |             |
| OH       | 10.3 (s)                  |             |             |
| OH       | 10.8 (s)                  |             |             |
| H2' and H6' | 8.0 (d, J = 8)            | 129.7       | 164.4 (C2); 161.5 (C4') |
| H5' and H5' | 6.90 (d, J = 8)           | 116.8       | 116.4 (C3' E 5'); 122.1 (C1'); 161.5 (C4') |
| H3'      | 6.8 (s)                   | 102.9       | 104.4 (C10); 122.1 (C1'); 164.4 (C2); 182.8 (C4) |
| H6'      | 6.28 (s)                  | 98.7        | 104.9 (C8); 160.9 (S); 163.1 (C7) |
| H1''     | 4.68 (d, J = 12)          | 74.1        | 71.4(C2'); 79.2 (C3'); 104.9 (C8); 156.4 (C9); 163.1 (C7) |
| H2''     | 3.8 (s)                   | 71.4        | 79.2        |
| H3''     | 3.26                     | 79.2        |             |
| H4''     | 3.3                      | 71.4        |             |
| H5''     | 3.25 (t)                 | 82.7        | 71.4        |
| H6''     | 3.7                     | 61.8        |             |
| H6''     | 3.5 (m)                  | 61.8        |             |

Chemical shifts are in ppm. All signals were assigned by 1H NMR, 13C NMR, HSQC, HMBC and COSY experiments. *Multiplicity.
ROESY (Rotating-frame Nuclear Overhauser Effect Spectroscopy) spectra were used to obtain information on the interaction between β-CD and the vitexin, and a close relation was observed between the symmetrical B-ring hydrogens of the flavone and the hydrogens H3 of the oligosaccharide, according to Figure 3. This interaction suggests that the B-ring of vitexin is included in the cavity of the host molecule, since there is interaction between the protons of the CD cavity (H3) [34].

3.2.2. Characterization by Scanning Electron Microscopy (SEM)

Figure 4 shows the photomicrographs of β-CD (a), vitexin (b), physical mixture-PM (c) and of β-CD/vitexin complex (d). For the free β-CD, rectangular surfaces of varied sizes and with fragments of adhered crystals were observed, as already presented by [35, 36]. Vitexin is presented as spheres of varying sizes and crystals, as previously reported by [37]. In PM (Figure 4c), vitexin (spheres) of β-CD (rectangles) are well

![Figure 1. 1H-NMR (DMSO, 400 MHz) spectrum of vitexin with expansion to the aromatic hydrogens region.](image1)

![Figure 2. Expansion of the 1H-NMR spectra of β-CD (peaks in red) and vitexin/β-CD complex (peaks in blue) (D2O, 400 MHz).](image2)
distinguished. However, for the complex (Figure 4d) crystalline aggregates with changes in particle sizes and morphology that are quite different from the free starting material are observed. Thus, the spherical morphology is no longer present. That suggests the formation of the complex. According to Lyra and colleagues [38], when a complex is formed, the resulting crystalline state is different from that obtained by simply mixing the host molecule with the CDs.

3.2.3. Infrared absorption spectrophotometry (FT-IR)

FT-IR is widely used to characterize the formation of inclusion complexes with CDs by detecting changes in the shape and position of absorbance bands across the vibrational spectrum of the different functional groups of the complexed or free drug molecules [38].

Figure 5 shows the FT-IR spectra of β-CD (a), vitexin (b), PM (c) and vitexin/β-CD IC (d). The spectrum reveals that the oligosaccharide shows typical absorptions of β-CD, with the signal around 1150 cm⁻¹ characteristic of saccharide structures, as it is attributed to pyranose ring vibration and the asymmetric stretching of C–O–H bonds [39]. The band around 3400 cm⁻¹ refers to the OH functional group.

The vitexin spectrum shows absorption bands corresponding to the functional groups –OH, C=C (aromatic), H=C (aromatic) and O–C (aromatic), respectively 3200-3650 cm⁻¹, 1450-1600 cm⁻¹, 3050-3150 cm⁻¹ and 1000-1300 cm⁻¹, as described by [40]. The absorption of conjugated carbonyl and intramolecular hydrogen bonding occurs approximately at 1665 cm⁻¹ and medium-sized signals between 1600 and 1500 cm⁻¹ also indicate the presence of aromatic groups in this molecule [41].

The spectrum of the physical mixture shows an overlap of the substances involved, and in some bands flavonoid predominates and in others the oligosaccharide predominates. The same is true of the complex in which the β-CD and vitexin bands predominate, and the main difference between the physical mixture and vitexin/β-CD IC is in the signal in 1155 cm⁻¹ that appears in the wider complex. The signal at 832 cm⁻¹ present in flavone refers to out-of-plane CH (aromatic) stretch [40] and the absence of this signal in vitexin/β-CD IC confirms the inclusion of vitexin B cavity of β-CD, as already verified in ROESY.

3.2.4. In vitro dissolution study

Figure 6 shows that in the first 3 min it is observed that the dissolution rate of free vitexin is 16.6% and that of the vitexin/β-CD complex is 47.2%, that is, the dissolution rate of vitexin in the vitexin/β-CD complex it is 3 times greater than for free vitexin. This 3 times higher dissolution rate for the complex remains at 6 min. In the 10 min the rate of dissolution of vitexin is approximately 4 times higher for the complex.

For free vitexin, 60% solubilization of this flavone was observed in the first 20 min, reaching a dissolution rate of 88.2% vitexin solubilized in 60 min. A different profile is observed for the vitexin/β-CD inclusion complex, and it is observed that in the first 20 min the solubilization of 73.3% of vitexin occurs, that is, 13.3% more when compared to free. In general, there is an increase in the solubility of vitexin within the first 30 min for the vitexin/β-CD complex than for the free vitexin. This shows that in the solution of the vitexin/β-CD complex there is a higher concentration of dissolved vitexin compared to free in the first 30 min.

![Figure 3. Expansion of the two-dimensional ROESY spectrum of vitexin/β-CD complex (D₂O, 400 MHz).](image-url)
Figure 4. Photomicrographs (1000 x zoom) of β-CD (a), vitexin (b), PM (c) and the complex of vitexin/β-CD (d).

Figure 5. FT-IR spectrum for (a) β-CD, (b) vitexin, (c) physical mixture and (d) vitexin/β-CD inclusion complex.
3.3. Evaluation of expectorant activity in mice

In some respiratory diseases, as asthma and chronic obstructive pulmonary disease (COPD), mucus clearance is impaired as consequence of its higher viscosity and elasticity. Changes in the hydration and biochemical constituents induces mucus accumulation in airway and can lead to inflammation, providing an environment for microbial growth and impairing symptoms and cough and dyspnea [42]. Thus, new drugs that are effective in changes the viscosity of mucus, by increasing the water secretion or modifying glycoprotein pattern, can be discovered using phenol red secretion assay [27, 43].

Considering that vitexin reduced mucus production in a murine ovalbumin-induced allergic asthma [14], the expectorant effect of this flavonoid was evaluated using the phenol red secretion in the airways of mice. As showed in the Figure 7, vitexin suspended in distilled water (at doses 0.2, 1 or 5 mg/kg) did not increase phenol red secretion (2.96 ± 0.52, 2.46 ± 0.42, 2.81 ± 0.27 μg/mL, respectively) when compared to H₂O treated mice (2.46 ± 0.27 μg/mL). On the other hand, vitexin/β-CD IC (at doses 0.2, 1 or 5 mg/kg) increased significantly phenol red secretion (4.04 ± 0.47, 3.69 ± 0.42, 4.56 ± 0.52 μg/mL, respectively), when compared to β-CD treated mice (2.12 ± 0.13 μg/mL), and phenol red secretion in vitexin/β-CD IC treated mice was significantly higher than free vitexin at same dose of 5 mg/kg. Moreover, vitexin/β-CD IC was more potent than guaifenesin, since it presented the same efficacy in dose 500 times smaller. The data indicating that vitexin/β-CD IC is shown to be a promising expectorant, since at low doses it was able to increase phenol red secretion and to improve mucus clearance.

The data above that indicate that the vitexin/β-CD complex is a promising expectorant suggest that the oligosaccharide improves the bioavailability and solubility of vitexin in aqueous medium, since this flavone has low water solubility. Cyclodextrins have been widely used in the development of pharmaceuticals, particularly because of their complexing properties, which provide increased solubility and a consequent increase in the dissolution rate of poorly soluble drugs, stability and reduction of irritations (gastric, dermal or ocular) [44].

In a study by [45], it was observed that another flavone, naringenin, has an expectorant activity, significantly increasing the phenol red secretion of the mouse trachea at doses of 30–67 mg/kg (oral), showing the potential of this class of secondary metabolites in the airways. However, further studies are needed to determine the expectorant mechanism of vitexin and others flavonoids.

3.4. Antitussive activity

Cough is a response to superior airways irritation that can become excessive, nonproductive, and hypersensitive in respiratory diseases. In these situations, cough is initiated by sensory pathways in the airways that are all vagal in origin and contain neurons detecting chemical or mechanical stimuli [46]. Since coughing can be evoked by reflex pathways that have remained remarkably unchanged among animal species, including rodents, pre-clinical assays has predictive value for new treatments and interventions [47]. Thus, citric acid inhalation induces cough in mice by stimulating the C fibers and is considered an effective methodology for assessing antitussive drugs [29, 48].

The evidence that vitexin also reduced pro-inflammatory Th2-specific cytokines on bronchoalveolar fluid and inhibited the secretion of ovalbumin-induced IgE on blood plasma [14], and these mediators are involved in cough exacerbation in asthma, the antitussive effect of this flavonoid was evaluated in mice. In this study, vitexin suspended in distilled water (at doses 0.2, 1 or 5 mg/kg) and vitexin/β-CD IC (at doses 1 or 5 mg/kg, but not at 0.2 mg/kg) reduced significantly cough frequency when compared to H₂O or β-CD treated mice, respectively, as showed in the Figure 8. Still, data shown that vitexin is more potent in to reduce cough than codeine, since presented efficacy in a dose 30-fold less.

The inhalation of citric acid aerosols activates the C or Aδ fibers of the airways and induces cough reflexes, probably through mast cell stimulation mediated by leukotriene and/or histamine pathways acting on C fibers. In addition, activation of C fibers can be mediated by tachykinins, since substance P is known to stimulate mast cells [29, 49]. C fibers are the most numerous vagal nociceptors in the bronchi and lungs and express the ionic channels of the membrane, including TRPV1. Transient potential receptors (TRPs) are ion channel proteins, some of which are expressed at the sensory nerve terminals of the airways and TRPV1 is a cationic channel present in the smooth muscle and epithelium of the respiratory tract, and one of the agents that activate it is citric acid [50, 51, 52].

Besides antiasthmatic effect, it was shown that vitexin reduced capsaicin-induced hyperalgesia, that depends of TRPV1 activation, and downregulated the expression of TRPV1 in isoflurane-induced neurotoxicity in rat brain [7, 53]. This suggests that possibly the potential of vitexin as an antitussive demonstrated in this study may be related in some way to activity of TRPV1 receptor.

The abovementioned relations about the mechanisms of action of vitexin deserve more detailed studies, but the potential of this secondary metabolite to reduce cough and promote expectorant activity, especially when complexed with β-CD, should be recognized.
toxic and when LD50 states that when LD50 is lower than 1000 μg/mL, it is considered as non-toxic. The toxicity of the samples can be determined in order to classify them according to the criteria established by [30], which are the negative control. The toxicity of the samples can be determined by calculating the LD50, because at the highest concentration used (1000 μg/mL), the larvae were all alive for both the vitexin and the complex and it can be concluded that the samples are non-toxic. The toxicity of the samples can be determined by calculating the LD50, because at the highest concentration used (1000 μg/mL), the larvae were all alive for both the vitexin and the complex and it can be concluded that the samples are non-toxic.

3.5. Toxicity with Artemia salina

In the study of toxicity with Artemia salina, it was not possible to calculate the LD50, because at the highest concentration used (1000 μg/mL), the larvae were all alive for both the vitexin and the complex and the negative control. The toxicity of the samples can be determined in order to classify them according to the criteria established by [30], which states that when LD50 is lower than 1000 μg/mL, it is considered to be toxic and when LD50 > 1000 μg/mL, it is considered as non-toxic.

Choo and collaborators [54] conducting diabetes induction studies in rats and mice showed that vitexin presented no signs of toxicity at the highest dose of 2 g/kg. The bioassay allows the evaluation of acute toxicity and is considered to be essential as a preliminary test in the study of compounds with potential biological activity [55].

4. Conclusion

Vitexin was isolated from Jatropha mutabilis for the first time and the formation of the β-CD complex could be evidenced by the NMR/ROESY, FT-IR, SEM techniques and in vitro dissolution study, characterizing the partial inclusion of vitexin in the cavity of this oligosaccharide. Only vitexin/β-CD IC showed expectorant activity on phenol red secretion model in mice. Both, vitexin suspended in distilled water and vitexin/β-CD IC reduced the cough frequency in mice exposed to citric acid. This work confirms the pharmacological potential of vitexin on the respiratory disease, demonstrating the expectorant and antitussive action for the complex in low doses. Further studies should be performed to evaluate the mechanisms of action involved.

Declarations

Author contribution statement

E.C. Costa: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

P.M.N. Menezes: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

L.A. de Araujo Reibeiro and F.S. Silva: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

R.L. de Almeida: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

A.P. de Oliveira: Performed the experiments; Contributed reagents, materials, analysis tools or data.

E.C. de Cruz Araujo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

L.A. Rolim: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

X.P. Nunes: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Additional information

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