Potential Therapeutic Effects of Underground Parts of *Kalanchoe gastonis-bonnieri* on Benign Prostatic Hyperplasia

**Antonio Palumbo,1 Livia Marques Casanova, 2 Maria Fernanda Paresqui Corrêa, 2 Nathalia Meireles Da Costa, 3 Luiz Eurico Nasciutti,1 and Sônia Soares Costa 2**

1Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil  
2Instituto de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil  
3Programa de Carcinogênesis Molecular, Centro de Pesquisas, Instituto Nacional do Câncer, 20231-050 Rio de Janeiro, RJ, Brazil

Correspondence should be addressed to Luiz Eurico Nasciutti; luiz.nasciutti@histo.ufrj.br and Sônia Soares Costa; sscostabh@gmail.com

Received 28 July 2018; Accepted 9 December 2018; Published 2 January 2019

Academic Editor: Ester Pagano

Copyright © 2019 Antonio Palumbo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Benign Prostatic Hyperplasia (BPH) affects mainly older men. It is estimated to affect 50% of 51-60-year-old men and 70% of 61-70-year-old men. BPH is a nonmalignant proliferation of epithelial and stromal cells of the prostate gland regions. Despite the use of conventional pharmacological therapy, herbal medicines are used in BPH therapy, and several mechanisms of action have been suggested based on their complex chemical composition. Considering the ethnomedicinal uses of *Kalanchoe gastonis-bonnieri* (KGB), we evaluated the inhibitory effects on the proliferation of stromal cells from primary benign prostatic hyperplasia (BPH) of four different aqueous extracts from this plant: underground parts from specimens in flower (T1 treatment), leaves from specimens in flower (T2 treatment), and flowers (T3 treatment) and leaves from specimens not in flower (T4 treatment). T1, T2, T3, and T4 treatments at 250 μg/ml for 72 hours inhibited BPH cells by 56.7%, 29.2%, 39.4%, and 13.5%, respectively, showing that the KGB underground parts extract (T1 treatment) was the most active. Our findings show that the extract of the KGB underground parts (150 and 250 μg/ml) stimulates important changes in the BPH cells, modulating crucial processes such as proliferation, viability, and apoptosis. HPLC-DAD-MS/MS analysis provided a tentative identification of glycosylated syringic acid derivatives, glycosylated forms of volatile compounds, and lignans in this extract. Finally, these results suggest that there is a potential therapeutic use for KGB in BPH, which could improve the clinical management of the disease.

1. Introduction

Benign Prostatic Hyperplasia (BPH) is a nonmalignant proliferation of epithelial and stromal cells of the prostate gland, causing an enlargement of the gland that may or may not be associated with lower urinary tract symptoms (LUTS) which affect the quality of life [1–4].

BPH affects mainly older men; and the prevalence increases with age. BPH is estimated to affect 50% of 51-60-year-old men, and this number reaches 80 to 90% for men over 80 years old [2, 5, 6].

Two antagonistic phenomena are involved in maintaining the normal size of the prostate: the rate of cell proliferation and apoptosis (cell death). In normal tissue, these ratios are similar for both the epithelial and stromal cells. However, in BPH there is an imbalance where the cell proliferation rate increases considerably more than the rate of the apoptosis process [4, 7, 8]. There are evidences that androgens, estrogen, growth factors, and neurotransmitters may play an important role in the etiology of BPH [3]. Additionally, scientific and clinical studies have shown that an inflammatory process may also influence the onset of this disease [2, 9–11].

Currently, six categories of drugs are used in the treatment of BPH: herbal agents, selective α-adrenergic blockers, inhibitors of the enzyme 5α-reductase, antimuscarinic agents, β3-adrenergic agonists, and, more recently, inhibitors...
of the enzyme phosphodiesterase type 5 [12–14]. Additionally, there are current evidences that nonsteroidal anti-inflammatory drugs (NSAID) can improve LUTS [15].

Herbal medicines are used in BPH therapy, and several mechanisms of action have been put forward based on the complex chemical composition present in plants. The presence of different substances acting on specific targets makes herbal medicines a relevant therapeutic strategy in the treatment of prostatic hyperplasia. The main herbal medicine used in the treatment of BPH is an extract of _Serenoa repens_ fruit (Arecaceae), popularly known as “saw palmetto” for which there is strong evidence of clinical efficacy [16–18].

Many natural products are also used for the improvement of physiological functions as well as the symptoms of BPH. Among them, the pollen extract Cernitin (Scutellaria baicalensis) has shown potential for BHP [16–18]. Besides, many medicinal plant species have been tested in vitro and in vivo after they have shown potential for BHP treatment [19]. Some secondary metabolites from plants have also shown promising results in vitro and in vivo, among them phenolic substances such as isoflavones, lignans, and the stilbene resveratrol [20–24].

_Kalanchoe gastonis-bonnieri_ Raym.-Hamet & H. Perrier (syn. _Kalanchoe adolphi-englertii_ Raym.-Hamet) is a medicinal herb from the family Crassulaceae. It is used in Latin American medicine as a vaginal contraceptive as well as in the treatment of genital-urinary and vaginal infections [25]. In a previous study we reported the isolation of the new flavonoid quercetin 3-O-α-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside, as well as vicenin-2, a C-glycosyl flavone, from the leaf extract of KGB [26]. We also demonstrated that aqueous extracts from _K. gastonis-bonnieri_ (KGB) are effective in controlling dental bacterial plaque and calculus in dogs [27].

Extracts from KGB have been shown to immobilize in dog, to clump together, and to promote structural changes in rat sperm [28]. The production and storage of seminal fluid and other components of semen are intrinsically linked to the functions of the prostate [29, 30]. Considering the ethnomedicinal use of KGB, this study aimed to determine the efficacy of this herb in the treatment of BPH, by using an in vitro model of BPH primary cell culture, particularly focusing on the main hallmarks related to the development of the disease.

### 2. Materials and Methods

#### 2.1. Plant Material

In this study, leaves were collected from specimens in flower and from specimens not in flower of _Kalanchoe gastonis-bonnieri_ cultivated in a residential garden in the city of Rio de Janeiro. Underground parts and flowers were also obtained from specimens growing in the same garden. A sample of a flowering specimen was identified and its voucher specimen (RGA 31592) is deposited in the Herbarium of the Botany Department at the Institute of Biology of the Federal University of Rio de Janeiro.

#### 2.2. Extraction

Fresh leaves from _K. gastonis-bonnieri_ (KGB) (average length of leaves: 15 cm) were rinsed with distilled water, cut into small pieces, and crushed in a blender. The extract obtained from the leaves of specimens not in flower was filtered and resulted in a clear yellow liquid. The color of the leaf extract of the specimens in flower was a salmon pink. The flowers were extracted by infusion with distilled water (20% w/w). The same procedure was applied for extracting the underground parts harvested from the other specimens in flower. Table 1 shows the mass of the different parts of the _K. gastonis-bonnieri_ specimens and the yield obtained from the extraction of each part. All the extracts were frozen, lyophilized, and kept in a freezer at -20°C.

#### 2.3. HPLC-DAD/MS/MS

High-Performance Liquid Chromatography analyses with a Diode Array Detector coupled to a Tandem Mass Spectrometry (HPLC-DAD/MS/MS) were carried out at the Center for Mass Spectrometry of Biomolecules-CEMBIO (IBCCF, UFRJ). The Prominence Shimadzu Liquid Chromatography system used was composed of an LC-20AD pump, a degasser system DGU-20A, and a DAD detector SPD-M20A, coupled to a Maxis Impact Q-TOF mass spectrometer (Bruker) equipped with an electrospray ionization (ESI) interface. An ODS-Hypersil reverse phase C-18 column (Thermo Scientific: 3 μm, 150 mm, 2.1 mm) protected by a precolumn of the same material (3 μm, 1 mm, and 2.1 mm) was used. The columns were maintained at 40°C.

A sample of the extract (4 mg/ml) from the underground parts of KGB was diluted in a mixture of water acidified with formic acid 0.1% and acetonitrile (19:1). The injection volume was 20 μl.

| Fresh plant material/mass (g) | Lyophilized Extract (g) | Yield % |
|-----------------------------|-------------------------|---------|
| Leaves of plants not in flower (113.9) | 23.8 | 2.1 |
| Leaves of plants in flower (149.5) | 2.9 | 1.9 |
| Underground parts of plants in flower (82.9) | 0.8 | 1.2 |
| Flowers (87.3) | 3.4 | 3.9 |
The mobile phase consisted of eluent A, water containing 0.1% formic acid (Sigma-Aldrich), and eluent B, acetonitrile (Merck) containing 0.1% formic acid (Sigma-Aldrich). The samples were run for 40 min at 0.3 ml/min, and the absorbance was monitored between 210 and 400 nm. The gradient used was as follows: 0–5 min (5–20% B), 5–11 min (20–22% B), 11–12 min (22–100% B), 12–26 min (100% B), and 26–40 min (100–5% B).

The Q-TOF mass spectrometer was operated in the negative ion mode using the following parameters: capillary voltage, 5000 V; endplate offset, -500 V; pressure of nebulizer, -3.0 mbar; drying gas temperature, 200°C; nitrogen was used as both the sheath and drying gas at a flow rate of 8.0 l/min. The mass range analyzed was set at m/z 50-1200 and collision energy at -5 eV. An external calibration solution (sodium formate 100 mM in water/isopropanol 1:1) was injected in the column and detected in the dead time ensuring mass accuracy throughout the chromatographic analysis. The elemental composition of the detected compounds was determined considering mass errors below 5 ppm. The data was processed using the Bruker Compass Data Analysis software.

2.4. BPH Cell. The stromal cell cultures were obtained from patients undergoing a clinical and histological diagnosis for BPH. BPH stromal cells were isolated according to previously described methods [31]. Briefly, prostate tissue was washed with phosphate-buffered saline (PBS) before being diced into approximately 1 mm³ pieces. The fragments were transferred to 10 ml dissociation flasks containing a solution of DMEM supplemented with 10% FBS and 1mg/ml of type I collagenase (Sigma, St Louis, MO). Tissue specimens were dissociated by constant stirring with a magnetic stir bar for 2-4 h at 37°C. The supernatant was frozen at 4°C and the remaining tissues were submitted to a new cycle of dissociation as described above. After that, the supernatants from the first and the second cycles were centrifuged and washed with balanced saline solution, without calcium and magnesium at 1200 RPM three times. The resulting cells were seeded in 25 cm² flasks and left to attach to a defined medium composed of supplemented DMEM (10% FBS, antibiotic/antimycotic mixture (Gibco): Penicillin 100 U/ml, Streptomycin 100 μg/ml, and Fungizone 25 μg/ml) and placed in a tissue culture incubator at 37°C in humidified air containing 5% CO₂. Cells were fed 3 times a week. At subconfluence (approximately 90% occupancy in each bottle) they were harvested using 0.05% trypsin/EDTA (both from Sigma) and replated.

2.5. BPH Cell Proliferation Assay. The cell proliferation assay was performed using 1x10⁴ BPH stromal cells per well in 96-well plates using Dulbecco’s Modified Eagles Medium (DMEM) containing 0.5% ethanol and 1% Fetal Bovine Serum (FBS). Cells were treated with the extract of the underground parts, extract of leaves from the specimens in flower and not in flower, and extract of flowers of KGB for 72 hours. The treated BPH stromal cells and controls were washed with PBS, fixed in a solution of 100% ethanol for 10 minutes, and then stained with 0.05% solution of crystal violet (Vetec) for 10 minutes. After staining, the cells were washed with distilled water and incubated in methanol for 5 minutes on a plate shaker, and the supernatant was collected. The absorbance was measured on an ELISA reader (iMARK BIO-RAD) at 570 nm.

In this assay, we evaluated the inhibitory effects of the four treatments (T1–T4) on cell proliferation of BPH stromal cells. The four treatments were the extract of the underground parts (T1), leaf extract of the flowering specimens (T2), extract of the flowers (T3), and leaf extract of the specimens not in flower (T4) at 250 μg/ml for 72 hours.

2.6. BPH Cell Viability Assay. The cytotoxic potential of KGB underground parts extract (T1) against BPH stromal cells was assessed by a quantitative MTT colorimetric assay. This assay is based on the reduction of MTT by the mitochondrial enzyme NADH dehydrogenase tetrazolium dye in blue violet crystals known as formazan to detect and determine cell proliferation and viability [32]. The supernatants were removed from each well and replaced by the sample T1 in quadruplicate wells except for the zero time where after removing the supernatant 100 μl MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 0.5 mg/ml, Sigma) was added. After addition of MTT, the culture plate was kept at 5% CO₂ and temperature at 37°C for three hours. After this time, MTT was removed and 100 μl of DMSO (Sigma) was added. The absorbance was read in an ELISA reader (BIO-RAD iMARK) at 570 nm. The sample procedure was repeated 72 hours after the addition of the treatment and control.

The absorbance (optical density) of the treatments was calculated and their values were subtracted from the values for the wells incubated only with DMEM. Then, the percentage of cell viability was expressed using the formula: sample value (DMEM, DMEM + FCS or T1)/mean value at time zero (T = 0) x 100%.

2.7. BPH Cell Apoptosis Assay. After trypsinization and centrifugation, 1x10⁵ cells were resuspended in 200 μl of propidium iodide solution (PBS, Triton X-100 0.1%, and propidium iodide 50 μg/ml, Sigma) and incubated on ice for 5 minutes. After the incubation period, cell death was measured by flow cytometry (FACScalibur Becton Dickinson) after acquiring 20,000 events. The excitation of the fluorochrome was measured using an argon laser with a wavelength of 488 nm and the emission was collected through a filter 630/22 nm.

2.8. Statistical Methods. All data represent the mean ± standard deviation values of three independent experiments. Differences between groups were analyzed using one-way ANOVA followed by the multiple comparison Newman-Keuls test. The value p <0.05 (*) was considered statistically significant.

3. Results and Discussion

Four aqueous extracts were prepared from leaves, flowers, and underground parts of K. gastonis-bonnieri (KGB) specimens. The yield of the extract from leaves collected from the specimens not in flower (2.1 %) was similar to that observed for leaves from flowering specimens (1.9 %), while the yields
Figure 1: Proliferation of BPH stromal cells treated with different extracts of Kalanchoe gastonis-bonnieri (KGB). (a) Graph representing the proliferation of cells with the different extracts (T1-T4) of KGB at 250 μg/ml after 72 h. * p<0.05, ** p<0.001. Data represent the mean ± standard deviation values of three independent experiments. (b) Percentage of BPH stromal cells after treatment with four different extracts (T1-T4) of KGB. Data represent the mean ± standard deviation values of three independent experiments.

from the underground parts from flowering specimens and flowers were 1.2 % and 3.9 %, respectively (Table 1).

We evaluated the inhibitory effects of the four KGB preparations on the proliferation of stromal cells from primary benign prostatic hyperplasia (PBH). Underground parts extract from the flowering specimens of KGB (T1 treatment), leaf extract of KGB from flowering specimens (T2 treatment), flowers extract from KGB (T3 treatment), and leaf extract of KGB not in flower (T4 treatment) were tested at 250 μg/ml for 72 hours. T1, T2, T3, and T4 treatments inhibited the cells proliferation by 56.7%, 29.2%, 39.4%, and 13.5%, respectively (Figure 1).

The best results were observed for the underground parts extract (T1 treatment), which encouraged the continuation of our experiments with this sample in order to corroborate the preliminary detection of its activity in BPH.

3.1. BPH Cell Viability Assay. The extract of KGB underground parts (T1 treatment) significantly reduced the viability of BPH stromal cells treated with 250 μg/ml, promoting decay of more than 50% cell viability (Figure 2).

3.2. BPH Cell Apoptosis Assay. In order to clarify whether the reduction in the number of BPH stromal cells observed after the treatment with KGB underground parts was due to a blockage in the proliferation activity of these cells or due to an induction of cell death, we performed an apoptosis assay to address this question.

The percentage of dead cells detected in the sub/G0 region of the cell cycle after 72 hours was significantly higher when the cells were seeded in the presence of T1 treatment in the concentrations of 150 μg/ml and 250 μg/ml (Figure 3(a)).

However, the activity was not dependent on the concentration used. We observed that both concentrations of the extract were able to suppress the progression of BPH cells along the cell cycle, with no significant difference between them. Additionally, Figure 3(b) shows that there was, in the control group, a distribution throughout the different cell cycle phases, including the S and G2/M phases, thus indicating a proliferating profile of the BPH stromal cells in the absence of the KGB treatment. On the other hand, the treatment with T1 showed that BPH stromal cells were almost exclusively at the sub/G0 phase of the cell cycle.

Despite numerous reports on the use of plants or derivatives of natural products of plant origin for the treatment of benign prostatic hyperplasia, these activities have not always been proven in pharmacological studies.

The inhibitory activity observed for KGB in BPH cell proliferation was very effective, since the KGB underground parts (T1) at the concentration of 150 μg/mL was able to drastically reduce the proliferation activity and the viability of BPH stromal cells in 72 hours. Moreover, the same treatment also induced a strong increase in the apoptosis rates of BPH stromal cells, since a large percent of these cells were restricted in the sub/G0 phase of the cell cycle, as revealed by the flow cytometry analysis. In addition, the cell cycle profile presented by BPH stromal cells after the treatment with the KGB underground parts corroborated the proliferation data, as the normal transition throughout the cell cycle was blocked by the KGB treatment. Therefore, these results that show a concomitant decrease in the cell viability and proliferation, combined with an induction of cell death by apoptosis, may reveal a beneficial role of KGB in combating the process of prostate growth that culminates in the development of BPH.

Our results with KGB underground parts are comparable with those observed for extracts from two plants clinically used to treat BHP. The first one, Pygeum africanum (Tadenan®), inhibits the proliferation of cultured human
prostatic myofibroblasts and fibroblasts as well as enhances apoptosis at concentrations from 25 to 100 μg/ml [33, 34]. *Serenoa repens* (Permixon®), the second one, revealed a tissue-selective action resulting in morphological changes and augmented apoptosis rates in addition to the inhibition of nuclear membrane bound 5α-reductase isoenzymes catalytic activity in prostate cells at the concentration of 10 μg/ml [35]. Additionally, an increment of Bax-to-Bcl-2 expression and caspase 3 activity, molecules involved in the apoptotic pathway, has already been documented in prostatic tissue samples from BPH symptomatic patients under treatment with Permixon for at least 3 months [36]. Also, an *in vitro* study showed that treatment with *S. repens* leads to the lightening of BPH symptoms due to antiproliferative and proapoptotic effects exerted on prostate epithelia and triggered by the downregulation of IGF-1 signaling pathway and induction of JNK [37]. Finally, BPH treatment with finasteride, the main therapeutic approach employed for this disease management, also demonstrated a mechanism exclusively observed in epithelial cells: caspase-dependent apoptosis initiation through activation of caspases 3 and 6 [38]. In fact, the success of the main treatment approaches (phytotherapeutic or conventional pharmacologic drugs) that are routinely used in the treatment of BPH are related to the control of BPH growth by reducing proliferation and inducing apoptosis [33, 34, 36]. Thus, the effects of KGB on BPH seem very interesting, since this disease is largely characterized by an imbalance between the proliferation and apoptosis [4, 7, 8, 11]. Furthermore, it was recently shown that KGB underground parts were able to abrogate the androgen signaling in prostate malignant cell lineages, besides inducing the apoptosis via caspase 8 activation, thus reinforcing the therapeutic potential of KGB in prostatic diseases [39].

Although the greater activity is in the subterranean parts from *K. gastonis-bonniere* and therefore could be a disadvantage for a phytomedicinal preparation due to the nonrenewable characteristics of this part of the plant, this succulent herb is a fast growing species that propagates easily by asexual reproduction [40].

### 3.3. Chemical Composition of KGB Underground Parts

The extract from the KGB underground parts had its chemical composition assessed by HPLC-DAD/MS/MS in the negative ion mode. As the TOF analyzer enables high-resolution mass measurements, with mass errors below 5 ppm, it was possible to infer the molecular formula of the major constituents detected in the KGB underground parts. The resulting chromatogram is shown in Figure 4, while data on the major compounds detected are summarized in Table 2.

Peak 1 (Rt 4.5 min; λmax 261 nm) presented the [M-H]− ion at m/z 359.0994 (C15H19O10) as base peak. MS/MS spectrum showed a fragment at m/z 197.0458 (C9H9O5), suggesting the loss of a hexose unity. This substance could possibly correspond to a glycosylated form of syringic acid such as syringate 4-O-β-glucopyranoside [41]. However, isomers of syringic acid cannot be ruled out. Peak 3 (Rt 5.2 min; λmax 282 nm) also showed a [M-H]− ion for which the molecular formula C15H19O10 was proposed and a similar fragment at m/z 197.0457. We hypothesize that this substance could correspond to a glycosyl ester of syringic acid. Syringic...
Table 2: Major chemical compounds in the extract of underground parts from *K. gastoni-bonnieri* by HPLC-DAD/MS/MS.

| Peak No. | Rt (min) | Molecular formula [M-H] | Measured m/z [M-H] | Calculated [M-H] | Error (ppm) | UV\(\lambda_{\text{max}}\) (nm) | MS/MS fragment ions | Proposed compound |
|----------|----------|-------------------------|--------------------|-------------------|-------------|----------------------|---------------------|-------------------|
| 1        | 4.5      | \(C_{15}H_{19}O_{10}\)  | 359.0986           | 359.0984          | -0.6        | 261                  | 297.0457           | Syringic acid hexoside |
| 2        | 4.9      | \(C_{15}H_{20}NO_{8}\) | 306.1194           | 306.1194          | 0           | n.d.                 | 205.0380; 161.0455 | Unknown           |
| 3        | 5.2      | \(C_{15}H_{19}O_{10}\) | 359.0996           | 359.0984          | -3.6        | 282                  | 197.0458; 239.0572 | Syringic acid hexoside |
| 4        | 5.5      | \(C_{15}H_{21}N_{2}O_{6}\) | 406.1732           | 406.1721          | 0.1         | 255                  | 307.1040           | Unknown           |
| 5        | 6.0      | \(C_{15}H_{21}O_{9}\)  | 357.1196           | 357.1191          | 1.3         | 273                  | 177.0556           | Unknown           |
| 6        | 6.2      | \(C_{15}H_{25}O_{10}\) | 381.1780           | 381.1766          | -3.5        | n.d.                 | 235.1896; 161.0458 | Alkyl diglycoside  |
| 7        | 7.3      | \(C_{15}H_{22}O_{10}\) | 415.1668           | 415.1610          | 1.9         | n.d.                 | 269.1037; 161.0456 | Benzyldiglycoside  |
| 8        | 8.0      | \(C_{15}H_{21}O_{10}\) | 395.1935           | 395.1923          | -3          | n.d.                 | 249.1352; 161.0461 | Alkyl diglycoside  |
| 9        | 8.2      | \(C_{10}H_{13}O_{11}\) | 521.2035           | 521.2028          | -1.2        | n.d.                 | 359.1507           | Glycosylated lignan |
| 10       | 8.5      | \(C_{10}H_{13}O_{11}\) | 521.2022           | 521.2028          | 1.3         | 283                  | 359.1508           | Glycosylated lignan |
acid β-D-glucopyranosyl ester has already been reported for leaves of *Kalanchoe pinnata* and a derivative of this substance was recently reported in the underground parts of the same species [42, 43]. Thus, peak 1 could correspond to a syringic acid glycosylated at the phenolic hydroxyl, having a free carboxyl moiety, and peak 3 to the same aglycone glycosylated at the carboxyl moiety. This is corroborated by their UV spectra, which correspond to those of the aforementioned substances and the order of elution, since an ester is less polar than a carboxylic acid.

Peak 6 (Rt 6.2 min) showed the [M-H]⁻ ion at m/z 381.1780 (C₁₆H₂₁O₉), with fragments at m/z 235.1196 (C₁₀H₁₅O₅) and
161.0458 (C₉H₈O₃) at the MS/MS spectrum, corresponding to the loss of a deoxyhexose (e.g., rhamnose) and a C₆H₄O (butanol) unit, respectively. Peak 7 (Rt 5.5 min) in its turn presented the [M-H]⁻ ion at m/z 415.1618 (C₁₀H₂₀O₁₀) and fragments at m/z 269.1037 (C₁₁H₁₇O₅) and 161.0456 (C₆H₄O₃), corresponding to the loss of a deoxyhexose moiety and a C₆H₄O (benzyl alcohol) unit. Peak 8 (Rt 8.0 min) gave a [M-H]⁻ ion at m/z 395.1935 and fragments at m/z 249.1352 (C₁₁H₁₇O₅) and 161.0461 (C₆H₄O₃), corresponding again to a loss of a deoxyhexose and a C₆H₄O (pentanol or methylbutanol) unity. Thus, peaks 6, 7, and 8 were tentatively attributed to glycosidically bound volatile substances, which are commonly found in plants [44–46]. Alcohol and monoterpenyl glycosides are found in the roots of plants from Rhodiola species, which also belong to the Crassulaceae family [47].

Peaks 9 (Rt 8.2 min) and 10 (Rt 8.5 min) presented the [M-H]⁻ ions at m/z 521.2022 and 521.2035, respectively. Both presented C₂₆H₃₅O₁₁ as molecular formula [M-H]⁻ and a MS/MS fragment at m/z 359.1508, indicative of the loss of a hexose unit. As we did not observe any further fragmentation of the aglycones, many structural possibilities were found for these substances. All the possible substances of natural origin with this molecular formula found in the SciFinder database belonged to the class of lignans, with several possible skeletons. Thus, we postulate that peaks 9 and 10 correspond to glycosylated lignans. In the underground parts of Kalanchoe pinnata, a glycosylated aryltetralin lignan was recently reported [43]. Lignans are also present in roots of Rhodiola species [48].

There are several reports on the activity of lignans in BPH and prostate cancer. For instance, a lignan-enriched extract of flaxseed (Beneflax®) was capable of improving LUTS in patients with BPH in a double-blind placebo-controlled clinical trial [23]. A similar extract from flax hulls prevented the development of testosterone propionate- (TP-) induced BPH in rats [49]. Also, secoisolariciresinol diglucoside, the major lignan in flaxseed, was able to inhibit BPH in TP-induced BPH in rats. Enterolactone, a metabolite of this substance, was shown to block the proliferation of a human prostate stromal cell line by a mechanism involving the G protein-coupled estrogen receptor 1 [50]. Furthermore, the lignans from the medicinal species Campylostropis hirtella (Fabaceae) were shown to inhibit prostate specific antigen and to decrease the androgen receptor expression in a prostate cancer cell lineage. The most potent of those lignans (dehydrodiconiferyl alcohol) was further investigated and exhibited proapoptotic effects in these cells [24].

It was not possible to identify peaks 2 (Rt 4.9 min), 4 (Rt 5.5 min), and 5 (Rt 6.0 min). We reported the molecular formulas considered most likely here, with the smallest possible errors. However, we do not discard other structural possibilities for these peaks.

4. Conclusion

The present results seem very encouraging, since they reveal a potential use of the underground parts of Kalanchoe gastonis-bonniere in the treatment of benign prostatic hyperplasia, a condition that causes significant chronic morbidity for men. Furthermore, the increment in the “phytotherapeutic products portfolio” currently available could improve the management of this disease, since a large number of natural compounds has been described as reliable, safe, and cost effective in the treatment of several diseases.

In addition, the main mechanisms related to KGB treatment seem to be the inhibition of the proliferation activity along with the induction of apoptosis.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

Maria Fernanda Paresqui Corrêa’s Current address is Instituto Nacional de Propriedade Industrial (INPI), 20090-910, Rio de Janeiro, RJ, Brazil

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Antonio Palumbo carried out the BPH assays, contributed with the data analysis and discussion and with the manuscript preparation. Livia Marques Casanova performed the HPLC-ESI-MS/MS analysis and data interpretation and contributed to the literature search and background information as well as with the manuscript preparation. Maria Fernanda Paresqui Corrêa carried out the KGB extraction and provided the samples for BPH assays. Nathalia Meireles Da Costa contributed with the flow cytometry experiments. Luiz Eurico Nasciutti supervised the BPH assays and contributed to the discussion of the data. Sônia Soares Costa supervised the phytochemical study, contributing to the discussion of the results and the manuscript preparation. Luiz Eurico Nasciutti and Sônia Soares Costa were responsible for the study design. All the authors read the final manuscript.

Acknowledgments

Special thanks are due to Mariana Neubarth Coelho for help with HPLC-DAD-MS/MS analysis and Eduardo Matos for skillful technical support with HPLC-DAD-MS/MS. Maria Fernanda Paresqui Corrêa (process: 141570/2007-5), Antonio Palumbo (process: 142318/2011-6), and Livia Marques Casanova (process: 140277/2013-7) were granted with Ph.D. fellowships from CNPq. Antonio Palumbo received a postdoctoral fellowship from Fundação do Câncer Ary Frazузio, Brazil (Oncobiology Program/IBqM/UFRJ/Brazil). Livia Marques Casanova currently receives a postdoctoral fellowship from CAPES (PNPD/process: 1723490).
References

[1] G. Corona, L. Vignozzi, G. Rastrelli et al., “Benign Prostatic Hyperplasia: A New Metabolic Disease of the Aging Male and Its Correlation with Sexual Dysfunctions,” International Journal of Endocrinology, vol. 2014, Article ID 329456, 14 pages, 2014.

[2] B. Fibbi, G. Penna, A. Morelli, L. Adorini, and M. Maggi, “Chronic inflammation in the pathogenesis of benign prostatic hyperplasia,” International Journal of Andrology, vol. 33, no. 3, pp. 475–488, 2010.

[3] C. G. Roehrborn, “Pathology of benign prostatic hyperplasia,” International Journal of Impotence Research, vol. 20, no. 3, pp. S11–S18, 2008.

[4] K. T. Foo, “Pathophysiology of clinical benign prostatic hyperplasia,” Asian Journal of Urology, vol. 4, no. 3, pp. 152–157, 2017.

[5] C. Vuichoud and K. R. Loughlin, “Benign prostatic hyperplasia: Epidemiology, economics and evaluation,” The Canadian Journal of Urology, vol. 22, pp. 6–15, 2015.

[6] K. B. Lim, "Epidemiology of clinical benign prostatic hyperplasia," Asian Journal of Urology, vol. 4, no. 3, pp. 148–151, 2017.

[7] A. Prajapati, S. Gupta, and B. Mistry, "Prostate stem cells in the development of benign prostate hyperplasia and prostate cancer: Emerging role and concepts," Biomed Res. Int., vol. 2013, 10 pages, 2013.

[8] L. Minutoli, M. Rinaldi, H. Marini et al., "Apoptotic Pathways Linked to Endocrine System as Potential Therapeutic Targets for Benign Prostatic Hyperplasia," International Journal of Molecular Sciences, vol. 17, no. 8, p. 1311, 2016.

[9] B. Chughthai, R. Lee, A. Te, and S. Kaplan, "Inflammation and benign prostatic hyperplasia: Clinical implications," Current Urology Reports, vol. 12, no. 4, pp. 274–277, 2011.

[10] G. Robert, A. Descazeaud, Y. Allory, F. Vacherot, and A. de la Taille, "Should We Investigate Prostatic Inflammation for the Management of Benign Prostatic Hyperplasia?" European Urology, Supplements, vol. 8, no. 13, pp. 879–886, 2009.

[11] B. Krušlin, D. Tomas, T. Džombeta, M. Milković-Periša, and M. Ulamec, "Inflammation in Prostatic Hyperplasia and Carcinoma—Basic Scientific Approach," Frontiers in Oncology, vol. 7, 2017.

[12] R. V. Nunes, J. Manzano, J. C. Truzzi, A. Nardi, A. Silvinato, and W. M. Bernardo, "Treatment of benign prostatic hyperplasia," Revista da Associação Médica Brasileira, vol. 63, no. 2, pp. 95–99, 2017.

[13] K. Gupta, M. Yezdani, T. Sotelo, and J. B. Aragon-Ching, "A synopsis of drugs currently in preclinical and early clinical development for the treatment of benign prostatic hyperplasia," Expert Opinion on Investigational Drugs, vol. 24, no. 8, pp. 1059–1073, 2015.

[14] C. De Nuncio and A. Tabaro, "Innovations in medical and surgical treatment," Nature Reviews Urology, vol. 12, no. 2, pp. 76–78, 2015.

[15] A. Kahokehr, R. Vather, A. Nixon, and A. G. Hill, "Non-steroidal anti-inflammatory drugs for lower urinary tract symptoms in benign prostatic hyperplasia: Systematic review and meta-analysis of randomized controlled trials," BJU International, vol. 113, no. 2, pp. 304–311, 2013.

[16] E. Pagano, M. Laudato, M. Griffo, and R. Capasso, "Phyotherapy of benign prostatic hyperplasia. A minireview," Phytotherapy Research, vol. 28, no. 7, pp. 949–955, 2014.

[17] O. Allkanjari and A. Vitalone, "What do we know about phytherapy of benign prostatic hyperplasia?" Life Sciences, vol. 126, pp. 42–56, 2015.

[18] A. Keehn, J. Taylor, and F. C. Lowe, "Phytotherapy for Benign Prostatic Hyperplasia," Current Urology Reports, vol. 17, no. 7, 2016.

[19] H. Azimi, A.-A. Khakhshir, I. Aghdaei, M. Fallah-Tafti, and M. Abdollahi, "A review of animal and human studies for management of benign prostatic hyperplasia with natural products: perspective of new pharmacological agents," Inflammation & Allergy—Drug Targets, vol. 11, no. 3, pp. 207–221, 2012.

[20] K.-S. Chung, S.-Y. Cheon, and H.-J. An, "Effects of resveratrol on benign prostatic hyperplasia by the regulation of inflammatory and apoptotic proteins," Journal of Natural Products, vol. 78, no. 4, pp. 689–694, 2015.

[21] C. Eleazu, K. Eleazu, and W. Kalu, "Management of benign prostatic hyperplasia: Could dietary polyphenols be an alternative to existing therapies?" Frontiers in Pharmacology, vol. 8, 2017.

[22] A. E. Katz, "Flavonoid and botanical approaches to prostate health," The Journal of Alternative and Complementary Medicine, vol. 8, no. 6, pp. 813–821, 2002.

[23] W. Zhang, X. Wang, Y. Liu et al., "Effects of dietary flavexed lignan extract on symptoms of benign prostatic hyperplasia," Journal of Medicinal Food, vol. 11, no. 2, pp. 207–214, 2008.

[24] H.-Y. Han, X.-H. Wang, N.-L. I. Wang, Y.-C. Wong, and X.-S. Yao, "Lignans isolated from Campylostropis hirtella (Franch.) Schindl. decreased prostate specific antigen and androgen receptor expression in LNCaP cells," Journal of Agricultural and Food Chemistry, vol. 56, no. 16, pp. 6928–6935, 2008.

[25] A. L. Oosoki, P. Lohr, M. Reiff et al., "Ethnobotanical literature survey of medicinal plants in the Dominican Republic used for women's health conditions," Journal of Ethnopharmacology, vol. 79, no. 3, pp. 285–298, 2002.

[26] S. S. Costa, M. F. P. Corrêa, and L. M. Casanova, "A new triglycerosyl flavonoid isolated from leaf juice of kalanchoe gastonis-bonnieri (crassulaceae)," Natural Product Communications (NPC), vol. 10, no. 3, pp. 433–436, 2015.

[27] S. L. Abdalla, S. S. Costa, M. A. Gioso et al., "Efficacy of a Kalanchoe gastonis-bonnieri extract to control bacterial biofilms and dental calculus in dogs," Pesquisa Veterinária Brasileira, vol. 37, no. 8, pp. 859–865, 2017.

[28] M. M. De la Luz Miranda-Beltrán, A. M. Puebla-Pérez, A. Guzmán-Sánchez, and L. Huacuja Ruiz, "Male rat infertility induction/spermatozoa and epididymal plasma abnormalities after oral administration of Kalanchoe gastonis bonnieri natural juice," Phytotherapy Research, vol. 17, no. 4, pp. 315–319, 2003.

[29] S. Nieto-Cerón, H. Vargas-López, M. Pérez-Albacete et al., "Analysis of cholinesterases in human prostate and sperm," Chemico-Biological Interactions, vol. 187, no. 1-3, pp. 432–435, 2010.

[30] Y. Gat, M. Gornish, M. Heiblum, and S. Joshua, "Reversal of benign prostate hyperplasia by selective occlusion of impaired venous drainage in the male reproductive system: Novel mechanism, new treatment," Andrologia, vol. 40, no. 5, pp. 273–281, 2008.

[31] A. Palumbo, L. B. Ferreira, P. A. V. Reis de Souza et al., "Extracellular matrix secreted by reactive stroma is a main inducer of pro-tumorigenic features on LNCaP prostate cancer cells," Cancer Letters, vol. 321, no. 1, pp. 55–64, 2012.

[32] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," Journal of Immunological Methods, vol. 65, no. 1-2, pp. 55–63, 1983.
[33] D. Boulbès, L. Soustelle, P. Costa et al., "Pygeum africanum extract inhibits proliferation of human cultured prostatic fibroblasts and myofibroblasts," BJU International, vol. 98, no. 5, pp. 1106–1113, 2006.

[34] M. T. Quiles, M. A. Arbós, A. Fraga, I. M. De Torres, J. Reventós, and J. Morote, "Antiproliferative and apoptotic effects of the herbal agent Pygeum africanum on cultured prostate stromal cells from patients with Benign Prostatic Hyperplasia (BPH)," The Prostate, vol. 70, no. 10, pp. 1044–1053, 2010.

[35] C. W. Bayne, M. Ross, F. Donnelly, and F. K. Habib, "The selectivity and specificity of the actions of the lipido-sterolic extract of serenoa repens (Permixon®) on the prostate," The Journal of Urology, vol. 164, no. 3, part 1, pp. 876–881, 2000.

[36] R. Vela-Navarrete, M. Escribano-Burgos, A. López Farré, J. García-Cardoso, F. Manzarbeitia, and C. Carrasco, "Serenoa repens treatment modifies Bax/Bcl-2 index expression and Caspase-3 activity in prostatic tissue from patients with benign prostatic hyperplasia," The Journal of Urology, vol. 173, no. 2, pp. 507–510, 2005.

[37] T. L. Wadsworth, J. M. Carroll, R. A. Mallinson, C. T. Roberts Jr., and C. E. Roselli, "Saw palmetto extract suppresses insulin-like growth factor-I signaling and induces stress-activated protein kinase/c-Jun N-terminal kinase phosphorylation in human prostate epithelial cells," Endocrinology, vol. 145, no. 7, pp. 3205–3214, 2004.

[38] A. Bozec, A. Ruffion, M. Decaussin et al., "Activation of caspases-3, -6, and -9 during finasteride treatment of benign prostatic hyperplasia," The Journal of Clinical Endocrinology & Metabolism, vol. 90, no. 1, pp. 17–25, 2005.

[39] N. Shamaladevi, S. Araki, D. A. Lyn et al., "The andean anticancer herbal product BIRM causes destabilization of androgen receptor and induces caspase-8 mediated-apoptosis in prostate cancer," Oncotarget, vol. 7, no. 51, pp. 84201–84213, 2016.

[40] H. M. Garces, C. E. Champagne, B. T. Townsley et al., "Evolution of asexual reproduction in leaves of the genus Kalanchoe," Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 39, pp. 15578–15583, 2007.

[41] K. Wolfram, J. Schmidlt, V. Wray, C. Milkowski, W. Schliemann, and D. Strack, "Profiling of phenylpropanoids in transgenic low-sinapine oilseed rape (Brassica napus)," Phytochemistry, vol. 71, no. 10, pp. 1076–1084, 2010.

[42] K. Furrer, M. Raith, R. Brenneisen et al., "Two new flavonol glycosides and a metabolite profile of bryophyllum pinnatum, a phytotherapeutic used in obstetrics and gynaecology," Planta Medica, vol. 79, no. 16, pp. 1565–1571, 2013.

[43] M. Cryer, K. Lane, M. Greer et al., "Isolation and identification of compounds from Kalanchoe pinnata having human alpha-herpesvirus and vaccinia virus antiviral activity," Pharmaceutical Biology, vol. 55, no. 1, pp. 1586–1591, 2017.

[44] A. Kilic, H. Kollmannsberger, and S. Nitz, "Glycosidically bound volatiles and flavor precursors in Laurus nobilis L.," Journal of Agricultural and Food Chemistry, vol. 53, no. 6, pp. 2231–2235, 2005.

[45] C.-C. Chyau, P.-T. Ko, C.-H. Chang, and J.-L. Mau, "Free and glycosidically bound aroma compounds in lychee (Litchi chinensis Sonn.)," Food Chemistry, vol. 80, no. 3, pp. 387–392, 2003.

[46] P. Wu, M.-C. Kuo, and C.-T. Ho, "Glycosidically Bound Aroma Compounds in Ginger (Zingiber officinale Roscoe)," Journal of Agricultural and Food Chemistry, vol. 38, no. 7, pp. 1553–1555, 1990.
