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# Influence of polyvinylpyrrolidone, microcrystalline cellulose and colloidal silicon dioxide on technological characteristics of a high-dose *Petiveria alliacea* tablet

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**ABSTRACT**

**Purpose:** *Petiveria alliacea* L. (Phytolaccaceae) is a perennial shrub used by its immunomodulatory, anticancerogenic and anti-inflammatory properties. This study determined the influence of polyvinylpyrrolidone (PVP), colloidal silicon dioxide (CSD) and microcrystalline cellulose (MC) on the technological characteristic of a high-dose *P. alliacea* tablet prepared by the wet granulation method.

**Methodology:** The botanical and pharmacognostic analysis of the plant material was firstly performed, followed by a $2^3$ factorial design considering three factors at two levels: (a) the binder (PVP) incorporated in formulation at 10% and 15% (w/w); (b) the compacting agent (CSD) added at 10% and 15% (w/w) and; (c) the diluent (MC) included at 7.33% and 12.46% (w/w). The analysis of pharmaceutical performance and the accelerated and long-term stability of the best prototype were also completed.

**Result and discussion:** The binder, compacting agent and the interaction binder/diluent had a significant impact on breaking force of high-dose *P. alliacea* tablet. The optimum formula was found to contain 15% (w/w) of CSD, 7.33% (w/w) of MC and 10% (w/w) of PVP. At these conditions, the tablet shows a breaking force of 77.96 N, a friability of 0.39%, a total phenol content of 1.30 mg/tablet and a maximum disintegration time of 6 min.

**Conclusions:** The use of adequate amounts of PVP, MC and CSD as per the factorial design allowed the preparation of a tablet suitable for administration, despite the inappropriate flow and compressibility properties of the *P. alliacea* powder.

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High-dose plant tablet; *P. alliacea*; formulation; binder; diluent



## Introduction

The World Health Organization recognizes the importance of medicinal plants on the basis to sustain their safety, effectiveness, and quality for human administration. Natural products have significantly contributed to the drug discovery and are often considered as alternatives to synthetic medications [1]. Stricter quality control of natural products has been regulated in several countries. These regulations involve botanical and sanitary control of the plant material as well as the design, formulation and production of pharmaceutical formulations [2].

*Petiveria alliacea* L. (Phytolaccaceae) is a perennial shrub indigenous to the Amazon Rainforest which also growths in the Caribbean, used in folk medicine, among others, by its immunomodulatory, anticancerogenic and anti-inflammatory properties [3]. Leaves and stems from this plant are frequently used by oral administration, as infusions, decoctions, salads or by liquefying or chewing them [3,4]. Despite its widespread use, these forms of administration have a lack of standardization and poor-quality presentation, thereby leading to inconsistence in doses, toxicological and pharmacological effects.

Formulation of *P. alliacea* into a modern pharmaceutical tablet dosage form could be an interesting alternative to counter these problems, by providing a greater patient acceptance, easier administration, prolonged shelf-life, accurate dosage and a minimal microbiological contamination compared with liquid forms [5]. However, the development and production of a high-dose plant tablet, is a complex and extensive technological challenge. Indeed, plant-derived materials are considered fine and poorly compressible. Additionally, they show prolonged disintegrate times; affecting the active metabolite release [5]. Some reports have addressed techniques to solve these problems, including the wet granulation with not aqueous solvents [6,7]. Although, the literature shows several studies using plant extracts [5–7] in tablet formulations, scarce investigations exist with powders from non-extracted plant tissues. Unlike extracts, powders from whole plant tissues have a higher chemical complexity as they contain a greater number of metabolites, which can negatively impact on the technological performance of pharmaceutical formulations. Considering that phytomedicine tablets usually contain a high dose of derived-plant materials, the use of appropriate excipients for formulation becomes a critical issue to produce a tablet suitable for administration [6].

The aim of this study was to evaluate the influence of the polyvinylpyrrolidone (PVP), the colloidal silicon dioxide (CSD) and the microcrystalline cellulose (MC) on the breaking force of a high-dose *P. alliacea* tablet using a $2^3$ factorial design in order to obtain the best prototype as well as to analyze its pharmaceutical performance. To our knowledge, the design and the formulation of a tablet containing a dried powder from *P. alliacea* stems and leaves as active principle has not been reported.

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## Materials and methods

### Characterization of plant material

#### Botanical analysis

Stems and leaves of *P. alliacea* L. were from the estate named 'La Rosita,' from Santiago de Cuba, Cuba and identified by Florentino Bermúdez, botanist of the 'Centro Oriental para Ecosistemas y Biodiversidad' in Santiago de Cuba and a specimen was deposited in the herbarium of this institution (voucher number: 4997).

#### Pharmacognostic analysis

***Phenolic content.*** Considering the important presence of polyphenols in the aerial parts of this species [8,9] and the fact that multiple medicinal activities of *P. alliacea* have been attributed to these molecules [10], it was considered the determination of the total phenol content (TPC) in the plant powder according to the method described in the British Pharmacopoeia [11]. TPC was expressed as a percentage regarding the dry weight of *P. alliacea* powder (w/w).

***Determination of ash and acid-insoluble ash.*** Total ash was determined using the method described in British Herbal Pharmacopoeia [12]. The compliance with the following criteria was considered: no more than 14% of total ash on the anhydrous basis and no more than 3% of acid-insoluble ash on the dried basis (insoluble in hydrochloric acid).

***Microbiological quality control of the plant material.*** Total bacterial and fungi as well as the presence of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* in *P. alliacea* powder were determined by methods recommended by the British Pharmacopoeia [11], performed in triplicate. The compliance with the criteria below was checked: (a) total viable aerobic count: no more than $2 \times 10^5$ colony-forming units (CFU) per gram (bacteria) and no more than $10^4$ CFU per gram (fungi); (b) absence of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*.

***Stability of the plant material.*** The *P. alliacea* powder was stored at room temperature ($30 \pm 2$ °C; $70 \pm 5$% relative humidity) during a year in a polyethylene bag. The organoleptic characteristics of color, odor and humidity as well as the TPC and the microbiological content were determined in the plant material.

***Density and compressibility determinations of the plant material.*** To predict the *P. alliacea* flow powder's characteristics, the Hausner Ratio (HR) and the closely related compressibility index (CI) were determined according to the methods described in United States Pharmacopoeia [13].

### Preparation of P. alliacea tablets

#### Excipients

Microcrystalline cellulose (MC 101, Gujarat Microwax, India), colloidal silicon dioxide (Aerosil V-200, Degussa AG, Hamburg, Germany), Polyvinylpyrrolidone (Kollidon-25, BASF, Germany), magnesium stearate (MS) (Otto Bärlocher GmbH, Munich, Germany) and ethylic alcohol (CAI, La Havana, Cuba) were used as received.

#### Tablet preparation

Before used, the plant material was washed and oven dried (temperature <35 °C; 5–6% humidity), then it was ground at a particle size ≤125 μm. The *P. alliacea* powder and the CSD were pre-mixed in weight proportion according to the factorial design (Table 1), ground in an ERWEKA Ball Mill, KM 5. MC and PVP previously passed by sieve No. 325 (45 μm) were mixed with the powder blend during 10 min and granulated in an ERWEKA Laboratory Kneader LK5 with 70% (v/v) ethanolic aqueous solution and ground in an ERWEKA Wet Granulator FGS with a sieve No. 10 (2.0 mm). Considering the poor flowability and compressibility characteristics of the plant material and its high proportion in the formulation, MC was granulated together with *P. alliacea* powder, CSD and PVP. The MC (grade 101) is recommended for wet granulation according to manufacturers. The MC 101 ensures distribution of the granulating fluid, leading to a uniform granulation, and drying. The granules were dried at 50 °C during 1 h in a Memmert oven. Then, the granule humidity was determined (3.5–4.5%) and the dried granules ground in an ERWEKA Dry Granulator TG 2000 to obtain 25% max. of fines. For lubrication, MS, previously passed through US sieve No. 325, was added and blended with the granules during 5 min at 15 rpm using an ERWEKA Double Cone Mixer DKM. This lubricated blend was compressed into tablets using a 0.5-inch (12.7 mm) diameter standard concave tooling in an ERWEKA 8-Station Rotary Tablet Press TR-D8. The applied compression force was adjusted to a fixed level of 1500 kg, the same for all the experiments. The nominal weight of tablets was 600 mg ± 5%.

Table 1. Factorial design and dependent variable (breaking force) used for the development of *P. alliacea* tablets.

| Exp | PVP % (w/w) (coded) | CSD % (w/w) (coded) | MCC % (w/w) (coded) | Breaking force (N) |
|---|---|---|---|---|
| 1 | 10 (−1) | 10 (−1) | 7.33 (−1) | 64.82 ± 5.88 |
| 2 | 15 (1) | 10 (−1) | 7.33 (−1) | 71.29 ± 3.82 |
| 3 | 10 (−1) | 15 (1) | 7.33 (−1) | 77.96 ± 6.57 |
| 4 | 15 (1) | 15 (1) | 7.33 (−1) | 72.76 ± 1.66 |
| 5 | 10 (−1) | 10 (−1) | 12.46 (1) | 62.07 ± 0.49 |
| 6 | 15 (1) | 10 (−1) | 12.46 (1) | 73.05 ± 0.78 |
| 7 | 10 (−1) | 15 (1) | 12.46 (1) | 71.39 ± 1.56 |
| 8 | 15 (1) | 15 (1) | 12.46 (1) | 79.33 ± 3.43 |

Exp: indicates the experimental run; PVP: polyvinylpyrrolidone; CSD: colloidal silicon dioxide; MCC: microcrystalline cellulose; PA: *Petiveria alliacea* powder; MS: magnesium stearate.

#### Experimental design

Taking into account that the tablet formulation using non-extracted plants as active material has several technical drawbacks such as an inappropriate compressibility that conspire against the final product quality, a $2^3$ factorial design was used for obtaining the best tablet formulation. Three factors each at two levels were evaluated: (a) the binder (PVP) incorporated into the powder mass at 10% and 15% (w/w); (b) the compacting agent (CSD) at 10% and 15% (w/w) and; (c) the diluent (MC) at 7.33% and 12.46% (w/w). Considering that CSD is more than a glidant, for its functions and the high proportions used in the formulation (10% and 15% w/w), it was called compacting agent. CSD favors the compaction [14] and tablet hardness by reducing interparticle friction and the formation of bridges and powder clumping. It is also known to form tablets with low friability, even at a low pression on the tableting machine [15]. To compare the effect of the factors, the values were coded (Table 1) and the breaking force (BF) of the tablets, considered as the dependent variable (response), was determined in all eight possible combinations. The resulting data were fitted into the SAS program 8.2 software (SAS Institute Inc., Cary, NC) and analyzed statistically using one-way ANOVA and means separated by Duncan's test.

### Characterization of the tablet prototype

#### Phenolic content of tablets

At least 10 tablets were triturated and the average weight was determined. The equivalent of 2 g of the drug (3000 mg of triturated tablets) was used and an extraction was performed with warm water (100 ml) and filtered. This solution (3 ml) was mixed with 2 ml water, 2 ml of phosphomolybdotungstic acid reagent acid reagent, 20% sodium carbonate solution (1 ml) and water (19 ml) and the absorbance was measured at 760 nm. The tannic acid was used as a reference substance. TPC was expressed as follows (mg/tablet):

$$T_{phen} = A_a \times W_{rs} \times A_w / A_{rs} \times W_a \times 5 \quad (1)$$

where $A_a$ is the absorbance *P. alliacea* extract obtained from tablets; $W_{rs}$ is the weight of the reference substance; $A_w$ is the average weight of tablets; $A_{rs}$ is the absorbance of the reference substance, $W_a$ is the weight of *P. alliacea* used for preparing the extract and 5 is a dilution factor. The TPC in the tablets was established to be between 0.92 and 1.72 mg/tab.

#### Uniformity of weight

Uniformity of weight was performed by weighing 20 tablets individually taken at random; calculating the average weight and comparing the individual tablet weight regarding to the average mass as specified in British pharmacopeia [11]. No more than two tablets deviate from the average mass by more than 5% and none should deviate by more than twice this percentage [11].

#### Breaking force

The breaking force of 10 randomly selected tablets was determined using an ERWEKA Tablet Hardness Tester TBH 100. Acceptance criteria for *P. alliacea*'s tablet breaking force was established as (39.22–78.45 N).

#### Friability

Friability of the tablet samples was measured using the ERWEKA friability tester TAR200, which complies with USP testing standards. Six grams of tablets were weighed, placed into the friabilator, and rotated at 25 rpm during 4 min. Tablets were removed from the equipment, gently brushed and collectively weighed. Friability was calculated according to the Eq. (2).

$$\text{Friability}\,(\%) = (\text{IW} - \text{FW})/\text{IW} \times 100 \quad (2)$$

Where IW is the initial weight and FW is the final weight. The weight loss of the tablets should be less than 1%.

#### Thickness and diameter

Thickness and diameter were determined using ten tablets randomly selected which were measured with a digital micrometer (MITUTOYO, 0–25 mm/0.01 mm). The diameter was established to be 12.70 ± 0.5 mm, whereas thickness was considered between 5.60 and 5.90 mm.

#### Disintegration time

Disintegration time of six randomly selected tablets was determined using an ERWEKA disintegration tester Type ZT 322 using degassed distilled water at 37 °C. Disintegration time for the tablet should not exceed 15 min [11].

#### Microbiological analysis

Total count of bacterial and fungi and the presence of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* were determined as specified in the British Pharmacopoeia [11], performed in triplicate. The compliance with the criteria below was verified: (a) total viable aerobic count: no more than $10^5$ colony-forming units (CFU) per gram (bacteria) and no more than $10^4$ CFU per gram (fungi); (b) absence of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*.

#### Stability of the tablets

Three batches of tablet prototypes were packed in bottles of high-density polyethylene, 60 ml of capacity, cap diameter 29 mm with liner and cover of the same material. They were monitored during two months at accelerating conditions of temperature and relative humidity (45 ± 2 °C/75 ± 5% RH) to check their stability in terms of: (a) organoleptic aspect, (b) breaking force, (c) disintegration time, (d) phenolic content and (e) microbiological contamination. The same parameters were also analyzed when the batches were stored at room temperature (30 ± 2 °C; 70 ± 5% RH) during a year.

## Results

### Characterization of the P. alliacea powder

Botanical analysis confirmed the identity of the plant. Preliminary TLC analysis of the *P. alliacea* powder indicated the presence of terpenes such as the isoarborinol and the products of its racemization (Rf 0.49–0.75) (data not shown). The presence of this compound in *P. alliacea* has been previously documented and it is considered as a marker of this plant [4,16]. The mean value for phenolic content for *P. alliacea* powder was 0.33%, whereas total and acid insoluble ash were determined to be 12.04% and 1.1% respectively. Mean values for total viable count of bacteria and fungus in the *P. alliacea* powder were $5 \times 10^4$ CFU/g and less than 10 CFU/g, respectively indicating a compliance with specifications (Table 2). It was not detected the presence of *Salmonella*, *Escherichia coli* or *Staphylococcus aureus* in the plant material. *P. alliacea* powder was stable during a year at room temperature (30 ± 2 °C; 70 ± 5% relative humidity) in a polyethylene bag (Table 2). The high values of the Carr index (36.9%) and Hausner's ratio (1.56) indicated that the powder had difficulty to flow. Consequently, the wet granulation method was selected for the tablet production.

Table 2. Stability of *P. alliacea* powder after one year in a polyethylene bag at room temperature (30 ± 2 °C; 70 ± 5% relative humidity).

| Time | Organoleptic properties | Humidity | Total polyphenol (mg/tablet) | Microbiological control |
|---|---|---|---|---|
| Initial | Color: Green<br>Odor: Ail | 8.5% | 0.33% | Total bacteria: $5 \times 10^4 \pm 0.71$ UFC/g<br>Fungi: <10 UFC/g<br>*Salmonella*, *Escherichia coli* or *Staphylococcus aureus*: Not detected |
| 1 year later | No changes were observed | 7.9% | 0.31% | Total bacteria: $10^5 \pm 1.41$ UFC/g<br>Fungi: <10 UFC/g<br>*Salmonella*, *Escherichia coli* or *Staphylococcus aureus*: Not detected |

### *Formulation study of the* P. alliacea *tablet*

Results from the $2^3$ factorial design are represented in Table 3. The binder (B), compacting agent (C) and the interaction binder–diluent (BD) had a significant influence on breaking force of *P. alliacea* tablet. Tertiary interaction binder–compacting–diluent (BCD) was not significant. As the interaction BD was significant, means were separated using Duncan's multiple range test [17]. Results of this test demonstrated that experimental runs 1, 2, 3, 4, 6 and 8 were statistically different from formulations 5 and 7 which showed lower breaking force values ($^*p < .05$).

### *Characterization of tablet prototype and stability studies*

Table 4 shows the composition of the tablet prototype. Biopharmaceutical parameters from this prototype met with quality requirements regarding to breaking force (77.96 N), friability (0.39%), tablet height (5.27 mm), disintegration time (6 min), uniformity of weight (average weight = 599.5 ± 2.56 mg), diameter (12.68 mm), total phenol content (1.30 mg/tablet) and microbiological analysis (bacteria: $1 \times 10^4$ CFU/g; fungi: 10 CFU/g). Results from the stability studies presented in Table 5 demonstrated that tablet prototype packed in bottles of high-density polyethylene was stable as no physical, chemical, or microbiological changes were observed throughout the accelerated and long-term stability studies.

**Table 3.** ANOVA of *P. alliacea* tablet's breaking force prepared as per $2^3$ factorial design.

| Source of variance | Degree of freedom | Sum of squares | Mean squares | *F* ratio | Significance |
|---|---|---|---|---|---|
| Treatments | 7 | 5.00548 | 0.71507 | 5.73 | $^*p < .05$ |
| B | 1 | 1.07123 | 1.07123 | 8.59 | $^*p < .05$ |
| C | 1 | 2.3716 | 2.3716 | 19.01 | $^{**}p < .01$ |
| D | 1 | 0.003025 | 0.003025 | 0.02 | $p > .05$ |
| BC | 1 | 0.5625 | 0.5625 | 4.51 | $p > .05$ |
| BD | 1 | 0.801025 | 0.801025 | 6.42 | $^*p < .05$ |
| CD | 1 | 0.0025 | 0.0025 | 0.02 | $p > .05$ |
| BCD | 1 | 0.1936 | 0.1936 | 1.55 | $p > .05$ |
| error | 8 | 0.9981 | 0.124763 | | |

Treatments: B: binder; C: compacting agent; D: diluent.

**Table 4.** Composition of the *P. alliacea* tablet prototype, 600 mg.

| Component | Function | Milligram per tablet | (% w/w) |
|---|---|---|---|
| Powder of Anamú (leaves and stems) | Drug substance | 400 | 66.67 |
| Colloidal silicon dioxide | Compacting agent | 90 | 15 |
| Microcrystalline cellulose | Diluent | 44 | 7.33 |
| Polyvinylpyrrolidone | Binder | 60 | 10 |
| Magnesium stearate | Lubricant | 6 | 1 |
| Total | | 600 | 100 |

## Discussion

In this study, the possibility of using a powder obtained from non-extracted leaves and stems of *P. alliacea* as an active ingredient for a new tablet development was analyzed. Assays were carried out to assess the impact of the PVP, CSD and MC on the breaking force of a high-dose *P. alliacea* tablet using a $2^3$ factorial design in order to obtain the best prototype as well as to analyze its pharmaceutical performance. This experimental approach allows to determine the relationships between factors affecting the development of a new formulation, being a useful statistical tool in the early stages of pharmaceutical development [18]. Farmacognostic analysis of plant material showed that the mean value of phenolic content was higher (0.33%) than that reported in a previous study for the soft extract from fresh leaves of *P. alliacea* (0.09%) [19]. This difference could be explained by a different plant provenance and by the presence of stems in the plant material. Values found for total and insoluble ash are in accordance with literature specifications and indicate a low content of inorganic and silicon contaminants [12]. High values of CI and HR suggested difficulties of the *P. alliacea* powder to flow and compression, likely due to its amorphous characteristics and the presence of sugars, which have insufficient flowability and compressibility properties [20]. For this reason, the wet granulation method was selected for tablet manufacturing. This method has the ability to incorporate high levels of active components by improving their flow and compression characteristics and by preventing the powder segregation [21]. Taking into account the stability data of the *P. alliacea* powder, bottles of high-density polyethylene with lid and crimp seals were proposed as primary packaging for tablets, to preserve the finished product against the humidity.

Regarding the *P. alliacea* tablet development, breaking force was determined to be the dependent variable for the factorial study, considering that mechanical resistance is a crucial point to be controlled when tablets contain a high dose of vegetal materials [5]. Indeed, *P. alliacea* powder showed deficient flow and compression, as per HR and CI values. These characteristics play a negative role to obtain a tablet with desirable physical–mechanical characteristics, being the breaking force a good parameter for determining when a tablet is strong enough to resist stresses caused by manufacturing process. As formulations 1, 2, 3, 4, 6 and 8 presented higher mechanical resistance, an economic analysis of these formulations was performed to choose the most promising prototype. Formulations 1 and 3 were firstly selected as they presented the lower levels of binder (PVP) and diluent (MC), so they had a good breaking force at lower cost. The high significance of the factor C (CSD) at $^{**}p < .01$ was considered for taking the final decision. Formulation 3 was then considered as the optimum tablet prototype. Other studies also report the use of excipients such as PVP and MC for tablet development using crude plant extracts [7,22]. Moreover, the importance of CSD in improving granule compactness containing a high proportion of spray-dried extracts

**Table 5.** Accelerated and long-term stability studies using the *P. alliacea* tablet prototype.

| Evaluated parameters | Batch 1 | | | Batch 2 | | | Batch 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Time (months) | 0 | 2 | 12 | 0 | 2 | 12 | 0 | 2 | 12 |
| Stability study | – | ASS | LTSS | – | ASS | LTSS | – | ASS | LTSS |
| Breaking force (N) | 65.31 | 57.85 | 55.89 | 64.33 | 57.36 | 47.95 | 57.17 | 54.91 | 48.83 |
| Disintegration time (min) | 3 | 1 | 1 | 3 | 1 | 1 | 3 | 1 | 1 |
| Phenolic content (mg/tab) | 1.3 | 1.31 | 1.31 | 1.3 | 1.33 | 1.29 | 1.28 | 1.31 | 1.28 |
| Microbiologic analysis | B: | B: | B: | B: | B: | B: | B: | B: | B: |
| B: CFU/g | $1 \times 10^4 \pm 0.71$ | $5 \times 10^4 \pm 1.41$ | $4 \times 10^4 \pm 1.41$ | $2 \times 10^4 \pm 0.71$ | $7 \times 10^4 \pm 2.12$ | $5 \times 10^4 \pm 1.41$ | $3 \times 10^4 \pm 2.12$ | $5 \times 10^4 \pm 1.41$ | $6 \times 10^4 \pm 0.71$ |
| F: CFU/g | F: < 10 | F: < 10 | F: < 10 | F: < 10 | F: < 10 | F: < 10 | F: < 10 | F: < 10 | F: < 10 |

ASS: accelerated stability study; LTSS: long-term stability study; B: bacteria; F: fungi.

from *Passiflora edulis* leaves has been demonstrated [23]. The compliance of tablet prototype with biopharmaceutical parameters demonstrated that this formulation was suitable for larger scale production. Stability studies indicated the suitability of the high-density polyethylene bottles as the primary package for the *P. alliacea* tablet during a year at ambient conditions. So, this period was proposed as the expiration time for the new formulation.

## Conclusions

The formulation of a tablet containing a high dose of *P. alliacea* was performed using a $2^3$ factorial design in which the influence of PVP, MC and CSD as binder, diluent and compacting agent on breaking force was analyzed as well as the pharmaceutical performance of the best prototype. The study demonstrated that the binder, compacting agent and the interaction binder/diluent had a significant impact on breaking force of *P. alliacea* tablet. The optimum formula was found to contain 15% (w/w) of CSD, 7.33% (w/w) of MC and 10% (w/w) of PVP. At these conditions, the tablet shows a breaking force of 77.96 N, a friability of 0.39%, a total phenol content of 1.30 mg/tablet and a maximum disintegration time of 6 min. As the disintegration time is not predictive of the *in vivo* performance of the *P. alliacea* tablet, further studies should be performed to evaluate the *in vitro* dissolution profile and the *in vivo* pharmacokinetic characteristics of this new solid dosage form.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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