

Research article

Seed germination, phenology, and antiedematogenic activity of *Peperomia pellucida* (L.) H. B. K.

Maria de Fátima Arrigoni-Blank*¹, Ricardo Luiz Barros Oliveira¹,
Sandra Santos Mendes¹, Paulo de Albuquerque Silva¹,
Ângelo Roberto Antonioli², Jeane Carvalho Vilar², Sócrates Cabral de
Holanda Cavalcanti² and Arie Fitzgerald Blank¹

Address: ¹Departamento de Engenharia Agrônômica, CCBS, Universidade Federal de Sergipe, Av. Marechal Rondon S/N, Jardim Rosa Elze, São Cristovão, SE, 49100-000, Brazil and ²Departamento de Fisiologia, CCBS, Universidade Federal de Sergipe, Av. Marechal Rondon S/N, Jardim Rosa Elze, São Cristovão, SE, 49100-000, Brazil

E-mail: Maria Arrigoni-Blank* - mfatima@zipmail.com.br; Ricardo Oliveira - rlbo@bol.com.br; Sandra Mendes - sandy-se@zipmail.com.br; Paulo Silva - pas77@ig.com.br; Ângelo Antonioli - aroberto@ufs.br; Jeane Vilar - jcvilar@bol.com.br; Sócrates Cavalcanti - schc@infonet.com.br; Arie Blank - afblank@ufs.br

*Corresponding author

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Abstract

Background: *Peperomia pellucida* is popularly known as *coraçãozinho* in the Brazilian northeast and is used in the treatment of abscesses, furuncles, and conjunctivitis. Our work aimed to determine the term of the development stages and the species cycle in the four seasons of the year (complete development, beginning of bloom, complete bloom, and seed set), verifying the plant's therapeutic profile during the four distinct development phases in order to detect differences in its potency. Pharmacological tests were performed to observe the anti-inflammatory activity.

Results: Phenological observations were accessed for a 12 month-period, from the Brazilian summer of 1999/2000 to fall 2000. On average the plantules' emergence occurred 15 days after seeding. All plantules grew in a similar manner up to 25 days after transplantation in all seasons. Starting on the 25th day, we observed faster growth during spring, with plants reaching a height of about 60 cm after 100 days of transplantation, unlike other seasons, in which plants reached heights of 40, 40, and 35 cm during winter, summer, and fall, respectively. The *P. pellucida* aqueous extract showed significant anti-inflammatory activity during phenophases 1 and 2 of winter and spring. Depending on the plant's phenophase there was variation in the potency of edema inhibition.

Conclusion: *P. pellucida* has a phenological cycle of approximately 100 days. It is recommended that the *P. pellucida* aqueous extract is used as an antiedematogenic only during phenophases 1 and 2 of winter and spring.

Background

Peperomia pellucida (L.) HBK (*Piperaceae*) is popularly

known in Brazilian northeast as *coraçãozinho*, *língua de sapo*, *herva-de-vidro* or *herva-de-jaboti*. It is a herbaceous plant

with succulent alternate and ovate leaves, with terminal and axillary efflorescences, at the opposite side from leaves, developing well in loose and humid soil by the tree shadows.[1]

In folk medicine, this species is employed on abscesses, furuncles, and skin sores, as well as eye inflammation (conjunctivitis). Literature data confirm the species antimicrobial [2] and analgesic[3] effects while other activities, such as anti-inflammatory, were not yet studied. Other therapeutic properties are also attributed to *P. pellucida* depending on the region. There are popular descriptions of *P. pellucida* to lower cholesterol levels (northeast), or used on proteinuria and as diuretic (Guyana).[4] Other species of *Peperomia* were found to have wound healing properties.[5] Phytochemical studies revealed the presence of dill-apiol and pellucidin A, in *Peperomia pellucida*. [6] There are several reports about purification of compounds from the *Peperomia* genus. [7-10] In order to develop cultivation techniques, medicinal plant's phenology is one of the first data to be obtained. The name phenology is defined as the study of the plants and animals seasonal rhythm, including their life cycle events or pharmacological activities during each season of the year. These rhythms are closely related to climate changes.[11] The superior plant's phenological events are the emergence, growth, induction, seed establishing and dormancy breakage, leaves production and fall, induction and development of floral gems, anthesis, fruits production and maturation, and seeds dispersion, as well as other phenomena.[12]

The right season to collect the herbs should be determined aiming, not only the amount of plant to be collected, but also a minimal amount of active principles, which is of extreme importance for the production of phytotherapeutics.

The only report of phenology involving medicinal plants was briefly described by Panizza.[13] The *Kalanchoe brasiliensis* is popularly used as an anti-inflammatory and validated by pharmacological studies.[13,14] However Santos et al,[15] studying the same species, verified that right after floration the plant did not possess any anti-inflammatory property, on the contrary, it stimulates the inflammation process. The active principle levels may vary as a function of the plant's developing stage and/or the edapho-climatic conditions where it grows. In view of these facts there is an urge for studying the phenological phenomena of species, with the goal to determine the level of active principles on all plant's stages, and in this way determine which season the plant presents the highest level of active principles. In order to perform this study, it is necessary to collect the plant's material during all development stages, submitting this material for pharmacolog-

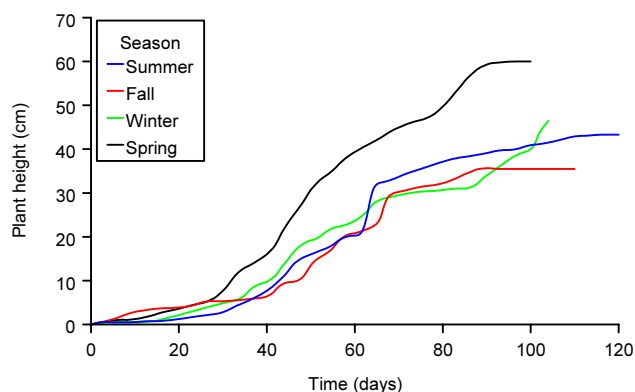


Figure 1
***Peperomia pellucida* seed germination in distilled water at room temperature.** Starting from the 4th day, when seeds initiated to germinate, until the 24th day where the germination rate reached 78%.

ical tests. Although phenology is a valuable scientific and economical knowledge, researches on this field are still scarce. However it is possible to find some reports, such as the *Lychnophora pinaster* Mart., [16] *Egletes viscosa*, [17] and *Mentha arvensis* L. var. *piperacens* Moor. [18]

In this work we wish to present a new approach to determine the pharmacological aspects of phenology. In other words, our goal was to determine the phenological stages span on all four seasons of the year, as well as, to verify the pharmacological activity during those development phases (complete development, beginning of bloom, complete bloom and seed set) through pharmacological tests. Using this methodology it is possible to confirm whether a plant's therapeutic value is modified or not during its development.

Results

Germination and phenology

The beginning of seed germination under our laboratory conditions occurred four days after seeding, where 78% germinated after 24 days (Figure 1).

Phenological observations were evaluated for a 12-month period, from summer 1999/2000 to spring 2000. Plantules emergence occurred 15 days after seeding, on average. The growth was similar for all plants up to 24 days after transplantation (DAT), in all seasons. Starting from the previous date, we observed faster increase during spring, when all plants were about 60 cm high after 100 DAT, against 40, 40, and 35 cm during winter, summer, and fall, respectively (Figure 2).

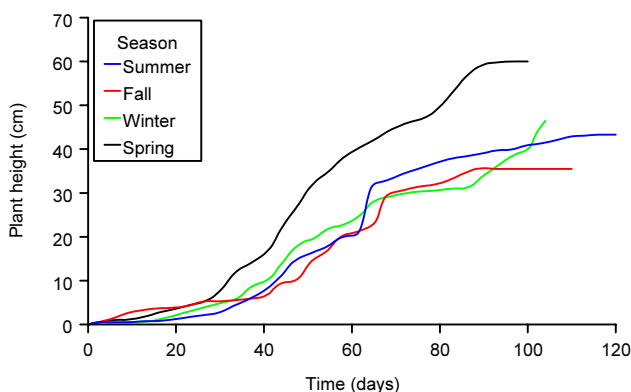


Figure 2
Peperomia pellucida height on four seasons of the year. The plants grow more during spring.

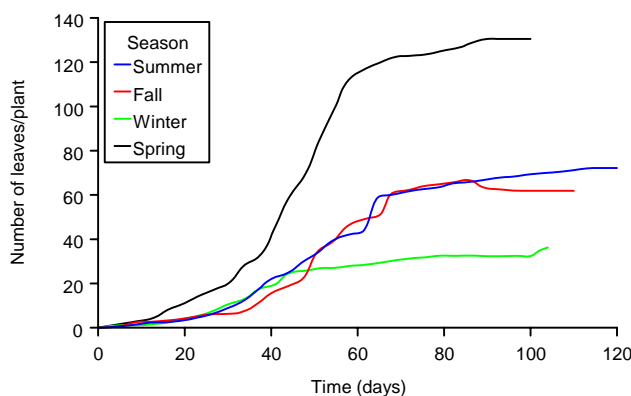


Figure 3
Number of leaves per plant on all four seasons of the year. Similar to their growth, the plants have more leaves during spring.

The cultivated species behaved in a different manner than the native one. Usually, native *P. pellucida* grows and develops during rainy periods (winter in Brazil's northeast). Growing under the shadows and in places rich in organic matter.

The number of leaves per plant was directly proportional to plant growth, obtaining an average of 130, 69, 62, and 32 leaves per plant during spring, summer, fall, and winter, respectively (Figure 3).

P. pellucida showed to have a relative short cycle, emerging two terminal and axillary efflorescences at the opposite side from leaves after 44 DAT during summer, 33 DAT during fall, 37 DAT during winter, and 27 DAT during spring (Figure 4).

Antiedematogenic activity

The *P. pellucida* aqueous extract showed antiedematogenic activity in all seasons of the year, which this study was performed, although the potency of edema inhibition was different, depending on the plant's phenophase (Tables 1 to 4). Plants collected during summer showed moderate edema inhibition during all phenophases, with values of 33, 34.5, 43.5, and 32% at the vegetative (phenophase 1), beginning of bloom (phenophase 2), complete bloom (phenophase 3), and seed set (phenophase 4), respectively. There were no significant differences between all four phases of development during summer (Table 1).

During fall, phenophases 2 (inhibition = 36.7%) and 4 (inhibition = 34.4%) showed the highest values of edema inhibition, however without substantial differences between both phenophases (Table 2). Phenophases 1 and 3 did not show statistically significant inhibition values.

Although *P. pellucida* plants grow on a slower rhythm during winter, the aqueous extract inhibited the rat paw edema mainly on phenophases 1, 2, and 4 with values of 41.6%, 36.7%, and 36.9% respectively. Phenophase 3 inhibited edema only by 21.1% (Table 3). The anti-inflammatory activity of plants cultivated during spring, also showed to be effective during phenophases 1 and 2, with inhibition values of 43% (phenophase 1), 42% (phenophase 2), 24.5% (phenophase 3), and 27.4% (phenophase 4, Table 4). Individual percentage values are shown below each paw volume in all tables. Total inhibition percentage is shown at the end of tables.

Discussion

Cultivation and phenology

By analyzing Figures 2 and 3 it is possible to notice that plants cultivated during winter, although growing to the same height as summer plants, presented half the number of leaves per plant. We suppose reduction in the number of leaves occurs because the plants are estiolated during this period, which is probably caused by higher cloudiness and less time of photoperiod.

During spring season, *P. pellucida* plants started the efflorescence earlier than other seasons. Generally about seven days after efflorescence the first fruits appear, which are small and drupe.[19] The fruits need seven more days to turn mature (brown) and able to disperse.

Winter was the least proficient period to cultivate this species, probably because of the rain excess and lack of luminosity in a screen protected environment. During this period, plants presented on average 13 flower stalks/plant after 100 DAT, while during spring this number increases to 89, during summer 37, and during fall 23 flower stalks/

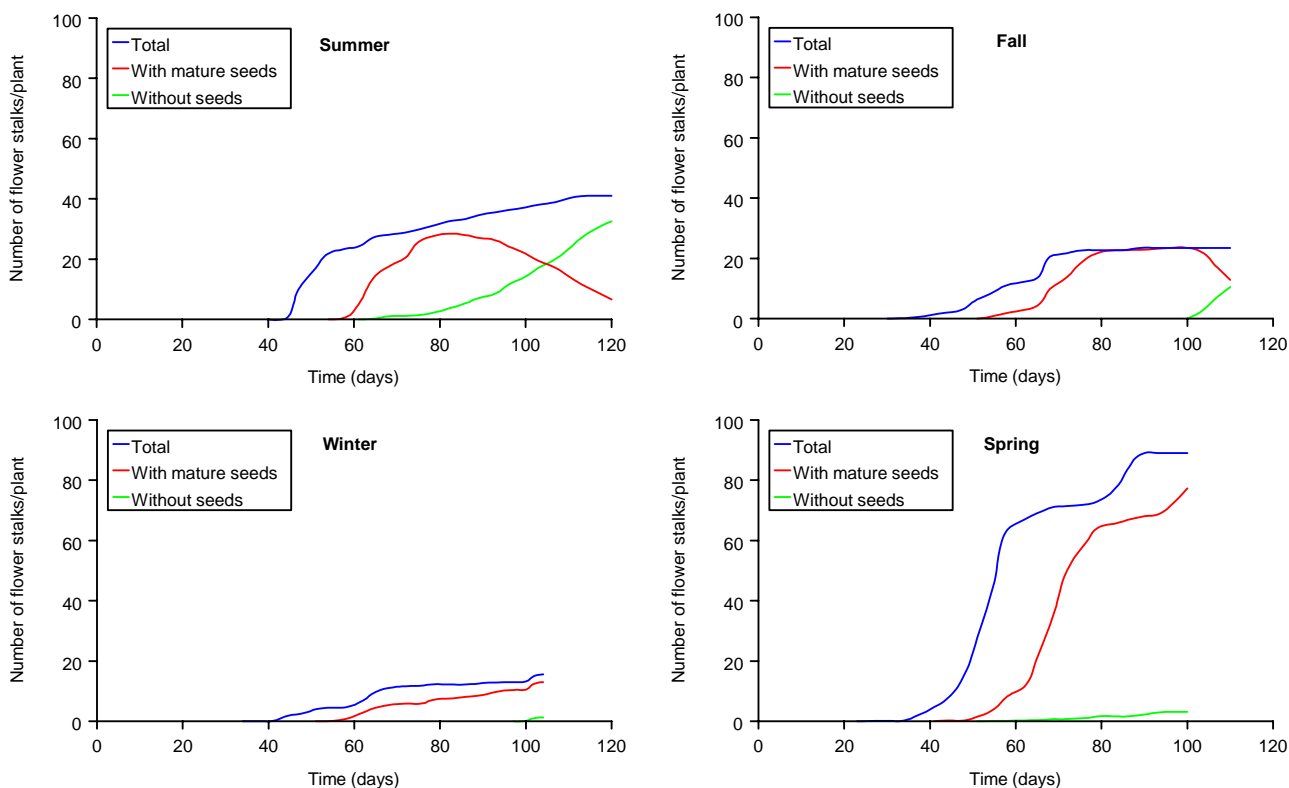


Figure 4
Number of flower stalks per plant during all four seasons of the year. The graphic shows the total number of flower stalks, as well as, flower stalks with and without seeds for a 120 days period.

Table 1: Effect of *Peperomia pellucida* aqueous extract (AE) p.o. on rat paw edema induced by carrageenan on different plant phases, during summer.

Treatment	Rat paw volumes after 1, 2, 3, and 4 h after Carrageenan injection (mL) mean ± SEM (individual % inhibition)				Edema % inhibition
	1 h	2 h	3 h	4 h	
Control	0.463 ± 0.037	0.731 ± 0.085	0.819 ± 0.062	0.642 ± 0.049	-
Indomethacin 10	0.080 ± 0.011** (82.7)	0.144 ± 0.018** (80.3)	0.232 ± 0.052** (71.7)	0.150 ± 0.059** (76.6)	77.2
Phenophase 1	0.297 ± 0.037 (35.9)	0.486 ± 0.097* (33.5)	0.516 ± 0.094* (37.0)	0.484 ± 0.082* (24.6)	33.0
Phenophase 2	0.372 ± 0.067 (19.7)	0.451 ± 0.039* (38.3)	0.514 ± 0.041* (37.2)	0.402 ± 0.038* (37.4)	34.5
Phenophase 3	0.249 ± 0.054 (46.2)	0.381 ± 0.051* (47.9)	0.471 ± 0.030* (42.5)	0.402 ± 0.035* (37.4)	43.5
Phenophase 4	0.370 ± 0.037 (20.1)	0.464 ± 0.030* (36.5)	0.561 ± 0.038* (31.5)	0.410 ± 0.032* (36.1)	32.0

Statistical significance: *p < 0.05, **p < 0.01 Vs control

Table 2: Effect of *Peperomia pellucida* aqueous extract (EA) p.o. on rat paw edema induced by carrageenan on different plant phases, during fall.

Treatment	Rat paw volumes after 1, 2, 3, and 4 h after Carrageenan injection (mL) mean \pm SEM (individual % inhibition)				Edema % inhibition
	1 h	2 h	3 h	4 h	
Control	0.463 \pm 0.037	0.731 \pm 0.085	0.819 \pm 0.062	0.642 \pm 0.049	-
Indomethacin 10	0.080 \pm 0.011** (82.7)	0.144 \pm 0.018** (80.3)	0.232 \pm 0.052** (71.7)	0.150 \pm 0.059** (76.6)	77.2
Phenophase 1	0.431 \pm 0.086 (6.9)	0.621 \pm 0.061 (15.0)	0.794 \pm 0.067 (3.1)	0.530 \pm 0.063 (17.4)	10.5
Phenophase 2	0.351 \pm 0.037 (24.2)	0.457 \pm 0.024 (37.5)	0.502 \pm 0.017** (38.7)	0.369 \pm 0.021* (42.5)	36.7
Phenophase 3	0.306 \pm 0.051* (33.9)	0.544 \pm 0.064 (25.6)	0.639 \pm 0.042* (22.0)	0.495 \pm 0.043 (22.9)	25.3
Phenophase 4	0.226 \pm 0.044** (51.2)	0.457 \pm 0.038* (37.5)	0.545 \pm 0.044** (33.5)	0.492 \pm 0.039 (23.4)	34.4

Statistical significance: *p < 0.05, **p < 0.01 Vs control

Table 3: Effect of *Peperomia pellucida* aqueous extract (EA) p.o. on rat paw edema induced by carrageenan on different plant phases, during winter.

Treatment	Rat paw volumes after 1, 2, 3, and 4 h after Carrageenan injection (mL) mean \pm SEM (individual % inhibition)				Edema % inhibition
	1 h	2 h	3 h	4 h	
Control	0.463 \pm 0.037	0.731 \pm 0.085	0.819 \pm 0.062	0.642 \pm 0.049	-
Indomethacin 10	0.080 \pm 0.011** (82.7)	0.144 \pm 0.018** (80.3)	0.232 \pm 0.052** (71.7)	0.150 \pm 0.059** (76.6)	77.2
Phenophase 1	0.232 \pm 0.063* (49.9)	0.355 \pm 0.035* (51.4)	0.552 \pm 0.038* (32.6)	0.399 \pm 0.036* (37.9)	42.0
Phenophase 2	0.241 \pm 0.039* (47.9)	0.469 \pm 0.040* (35.8)	0.547 \pm 0.024* (33.2)	0.442 \pm 0.031* (31.2)	36.7
Phenophase 3	0.412 \pm 0.035 (11.0)	0.532 \pm 0.050 (27.2)	0.621 \pm 0.066 (24.2)	0.527 \pm 0.047 (17.9)	21.1
Phenophase 4	0.257 \pm 0.064 (44.5)	0.414 \pm 0.074* (43.5)	0.355 \pm 0.061* (56.7)	0.469 \pm 0.066 (26.9)	36.9

Statistical significance: *p < 0.05, **p < 0.01 Vs control

Table 4: Effect of *Peperomia pellucida* aqueous extract (EA) p.o. on rat paw edema induced by carrageenan on different plant phases, during spring.

Treatment	Rat paw volumes after 1, 2, 3, and 4 h after Carrageenan injection (mL) mean \pm SEM (individual % inhibition)				Edema % inhibition
	1 h	2 h	3 h	4 h	
Control	0.463 \pm 0.037	0.731 \pm 0.085	0.819 \pm 0.062	0.642 \pm 0.049	-
Indomethacin 10	0.080 \pm 0.011** (82.7)	0.144 \pm 0.018** (80.3)	0.232 \pm 0.052** (71.7)	0.150 \pm 0.059** (76.6)	77.2
Phenophase 1	0.239 \pm 0.042** (48.4)	0.421 \pm 0.041** (42.4)	0.450 \pm 0.032** (45.1)	0.401 \pm 0.034* (37.5)	43.1
Phenophase 2	0.267 \pm 0.033** (42.3)	0.392 \pm 0.022** (46.4)	0.524 \pm 0.026** (36.0)	0.365 \pm 0.041* (43.1)	41.6
Phenophase 3	0.332 \pm 0.050 (28.3)	0.557 \pm 0.040 (23.8)	0.595 \pm 0.033** (27.4)	0.519 \pm 0.040 (19.2)	24.5
Phenophase 4	0.412 \pm 0.076 (11.0)	0.540 \pm 0.032* (26.1)	0.522 \pm 0.028** (36.3)	0.451 \pm 0.030* (29.8)	27.4

Statistical significance: *p < 0.05, **p < 0.01 Vs control

plant. It is important to mention that during some period the plants exhibit raw seeds, ripe seed, and flower stalks without seeds, which seeds have been already fallen.

Since the plant's leaves are used on infusions or may be used in a phytotherapeutic formulation, the number of leaves per plant should be taken into account when producing large quantities of dry plant material. Spring represents the most productive season. In order to verify the viability to produce *P. pellucida* leaves during other seasons a financial study should be performed.

Anti-inflammatory activity

The *P. pellucida* aqueous extract inhibited the inflammation process during all phenophases, although not as much as indomethacin. As shown on Table 1, during summer there was no statistically significant edema inhibition induced by carrageenan at the first hour of experiment for all phenophases. However during the second, third, and fourth hours there were moderate edema inhibitions (Table 1). The rat paw edema experiment is a classical model of acute inflammation used in the study of non-steroidal anti-inflammatory drugs involving various types of chemical mediators of inflammation, such as histamine, serotonin, bradykinin, and prostaglandines.[20,21] At the first hour of inhibition histamine is the major mediator found in the inflammation process. Experimental data from Table 1 suggest the *P. pellucida* aqueous extract is not involved with the histamine system to act as an antiedematogenic drug.

Table 2 shows the experiments conducted with plants cultivated and collected during fall. It was not detected statistically significant inhibition during phenophase 1, suggesting a lower concentration of antiedematogenic compounds in the plant during this phase. Nonetheless phenophases 3 and 4 show inhibition of the edema at the first hour. These results suggest that several compounds might be involved on the antiedematogenic process. Phenophase 4 showed higher inhibition values.

During winter (Table 3) the *P. pellucida* aqueous extract showed significant antiedematogenic activity during phenophases 1 and 2 starting from the first hour of inflammation. Plants collected during phenophase 3 did not show significant edema inhibition, however during phenophase 4 the antiedematogenic effect was observed only at the second and third hours of experiment. The experiments suggest that *P. pellucida* has different concentrations of chemical agents responsible for the antiedematogenic effect. In view of these facts the best periods to collect the plant to use as an antiedematogenic agent are phenophases 1 and 2 of winter and spring. We may consider as anti-inflammatory any substance, which inhibits the carrageenan effects, such as the aqueous extract of *P. pellucida*

collected during phenophases with highest values of inhibition.

Conclusions

P. pellucida possesses a phenological cycle of approximately 100 days. The aqueous extract showed antiedematogenic effect especially during phenophases 1 and 2 of winter and spring. *P. pellucida* shows antiedematogenic on some development phases, other phenophases show little activity. It is only recommended to use the *P. pellucida* extract as anti-inflammatory during phenophases 1 and 2 of winter and spring. More investigation is being conducted to find out which compounds are responsible for the antiedematogenic activity.

Material and methods

Cultivation and phenological studies

Seeds were collected by Dr. Arie Fitzgerald Blank from native *Peperomia pellucida* plants in our campus, followed by their processing and germination tests. The species exsiccate was identified by our herbalist (Gilvane V. Souza, Biology Department) and deposited in our University's herbarium under voucher number 03229.

Seeds germinative capacity was determined using four Petri dishes, loading 100 seeds in each dish, and keeping with distilled water under room temperature. Since north-east of Brazil is a tropical zone and this species is native in this region, there was no need to control temperature and humidity.

In order to perform the phenological assay, seedlings were produced in polyethylene trays (30 × 40 × 8 cm), kept under a 70% black screen, in our campus, using a mixture of vegetal earth and bovine manure (1:1) as substrate. Seedlings were transplanted to three polyethylene trays (30 × 40 × 8 cm) when they were about 1 cm high, using the same substrate above mentioned, adding only six plants per tray. The assays were performed in the same environment above mentioned. Data collection was performed twice a week from December 1999 to November 2000, comprehending the Brazilian summer of 1999/2000 and fall, winter, and spring of 2000. The average temperatures during summer and winter are 30°C and 25°C, respectively. The following plant characteristics were evaluated: plant height, number of leaves per plant, number of total efflorescence per plant, number of flower stalks with mature seeds, and number of flower stalks without seeds. On each season we performed the cultivation and conduction of the assays as described before.

Pharmacological tests were performed using another set of *P. pellucida* plants cultivated under the same conditions above mentioned. In order to verify the changes on biological activity during the seasons and phases we per-

formed activity tests during the following phenophases: vegetative (complete development), beginning of bloom, complete bloom, and seed set.

Aqueous extract preparation

For each phenophase the plant's leaves were dried at 40° C in a forced air oven (Marconi MA 037) and triturated using a mill in order to obtain a powder. Distilled water (1:10 w/v) at 100° C was added to the triturated powder, constituting the aqueous extract. The extract was infused for 30 min, filtered and lyophilized and used on pharmacological tests. The yield of the aqueous extract was 9.5% w/w. In order to perform all experiments the extract was reconstituted in enough water to make a 100 mg/mL solution and administered p.o. 60 minutes before the experiment.

Animals

Wistar rats (120-200 g) male and female were used as test animals. The animals were maintained in plastic boxes, with food and water *ad libitum*. The animals submitted to oral administration of the extract or drugs were fasted for 12 hours.

Drugs preparation

Drugs used in the experiments were diluted in a way to obtain an injection volume of 0.1 mL/10 g (animal weight), except when defined in the text. Each drug was dissolved in appropriate solvents as follows: Indomethacin (Sigma), diluted in water/0.1 N NaOH (pH = 8); carrageenan 1% (Sigma), in saline solution.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated using the rat paw edema test induced by carrageenan, using Wistar rats, according to the methodology of Winter *et al.*[22] Eight Wistar rats received, p.o., indomethacin (10 mg/Kg) as positive control, and other groups of eight rats received the *P. pellucida* aqueous extract (400 mg/Kg) for all four distinct phenophases, 1 h before subplantar injection of carrageenan (0.1 mL/paw, 1% solution). The aqueous extract concentration was determined according to a previous publication.[23] The paw volume was measured at the time 0, then measured again 1, 2, 3, and 4 h after carrageenan administration, by the water displacement method measured with the help of a plethysmometer (model 7150, Ugo Basile).

Statistical analysis

The inflammation test results were analyzed by ANOVA followed by the Tukey test and expressed as an mean \pm standard error mean (SEM). Inhibition percents were calculated by the formula: % edema inhibition = $(1 - V_t/V_c) \times 100$, where V_t and V_c represent the average paw volume of the treated and control groups, respectively. Individual

percentages were also computed and are show below rat paw volumes.

Authors' contributions

Author 1, M. F. Arrigoni-Blank, conceived of the phenology study and participated in its design and coordination. Author 2, R. L. B. Oliveira, carried out cultivation techniques. Author 3, S. S. Mendes, carried out phenology studies. Author 4, P. A. Silva, carried out germination studies. Author 5, A. R. Antonioli, conceived of the pharmacological studies and participated in its design and coordination. Author 6, J. C. Vilar, carried out the pharmacological assays. Author 7, S. C. H. Cavalcanti, carried out the statistical analysis and confection of the manuscript. Author 8, A. F. Blank, conceived and coordinated of the cultivation techniques.

All authors read and approved the final manuscript.

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