

Full Length Research Paper

## The *in vitro*, *ex vivo*, and *in vivo* experimental findings of *Nephrolepis exaltata*

Eduardo Matheus Ricciardi Suzuki<sup>1</sup>, David Freitas Fernandes Corrêa<sup>2</sup>,  
Isadora Caruso Fontana Oliveira<sup>1</sup>, Fâni Ribeiro Silva<sup>1</sup>, Regina Yuri Hashimoto Miura<sup>2</sup>,  
Flávia Aparecida Resende Nogueira<sup>3</sup>, Edson Hideaki Yoshida<sup>1</sup>, Magali Glauzer Silva<sup>1</sup>,  
Sandro Rostelato-Ferreira<sup>2</sup> and Yoko Oshima-Franco<sup>1\*</sup>

<sup>1</sup>Pharmaceutical Sciences, University of Sorocaba (UNISO), Sorocaba, SP, Brazil.

<sup>2</sup>Biological Sciences Course, Health Sciences Institute, Paulista University (UNIP), Sorocaba, SP, Brazil.

<sup>3</sup>Department of Biological Sciences and Health, University of Araraquara (UNIARA), Araraquara, SP, Brazil.

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***Nephrolepis exaltata* (L.) Schott decreases the heartbeats of cockroaches and it was postulated that the plant could be an anticholinesterase agent and could have effects. It was performed: (a) *In vitro*: hydroalcoholic extract of *N. exaltata* was pharmacognostically characterized, the cholinesterase activity determined with 1.0 and 3.0 mg/ml, comparing to positive control and negative control, and the preliminary toxicity was evaluated with 5 mg/plate through *Salmonella*/microsome assay using TA100 strain; (b) *Ex vivo*: 2, 5, or 10 mg of extract was assayed on mouse phrenic nerve-diaphragm preparation using conventional myographic technique; and (c) *In vivo*: 2.0, 1.0 or 0.5 g of extract was exposed to *Allium cepa* root cells, using onions bulbs for further measuring and microscopic analysis. The cholinesterase activities (U/L, n=3) of 1.0 and 3.0 mg/mL fern extract were of 2,866.6 ± 200.7 and 3,092.9 ± 214.2, respectively, versus 87.1 ± 58.1 (p<0.05) for positive control. The extract showed the absence of micronucleus and inhibited the root growth reaching 100% at 2 mg. The plant has no anticholinesterase activity, it is not toxic on bacterial reverse mutation or nerve-muscle parameters and is not genotoxic on *A. cepa* assay, but inhibits the root growth of *A. cepa*.**

**Key words:** *Allium cepa*, cholinesterase, fern, pharmacognosy, *Salmonella*.

### INTRODUCTION

*Nephrolepis exaltata* (L.) Schott, family Nephrolepidaceae, is an ornamental and perennial plant (Figure 1S), terrestrial or epiphytic in its native state, and is originally from the south of the USA – popularly known

as Boston fern, Central and South America. It is also naturalized in Canary Islands, Africa, Asia, India, Polynesia, and New Zealand (Large and Farrington, 2016). The plant has been studied for its soil

\*Corresponding author. E-mail: [yoko.franco@prof.uniso.br](mailto:yoko.franco@prof.uniso.br). Tel: +55 15 2101-7197; Fax: +55 15-2101-7112.

phytoremediation properties (Sultana et al., 2015) against arsenic and other metals (Osusu-Dentaah, 2017; Rao and Khan, 2017) as well as the antimicrobial effects of its volatile oil such as 2,4-Hexadien-1-ol (16.1%), nonanal (14.4%),  $\beta$ -Ionone (6.7%) and thymol (2.7%) (El-Tantawy et al., 2016). It has also been studied for its hormonal and cytotoxic effects on human cancer cells (Thomas-Charles and Fennell, 2019); its air-purifying capabilities (Chauhan et al., 2017), and its ability in hydrolyzing the ester linkages of phenol and aromatic and aliphatic alcohol acetates in biotransformation reactions (Mironowicz et al., 1994).

Faced with the crescent interest in studying this plant by the above advantages, Sanchez et al. (2018) described an insect-based model, in which aqueous extract of *N. exaltata* caused a decrease in heartbeats of a semi-isolated heart preparation of *Nauphoeta cinerea*. Researchers have postulated a possible insecticidal activity mediated by parasympathetic nerves at heart, where acetylcholine causes bradycardia due to blockade of postjunctional muscarinic receptors (Pace and Serpell, 2015). It is known that the standard battery for determining genotoxicity includes the assessment of mutagenic damage, using bacterial reverse gene mutation assay to detect relevant genetic changes as well as most genotoxic carcinogens for rodents and humans (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Thus, aiming to secure safety to *N. exaltata* plant, genotoxic studies were carried out by *Allium cepa* assay, an ideal bioindicator for the first screening of genotoxicity (Ramos et al., 2020), and also toxicity determination by *Salmonella*/microsome assay. Besides, since organophosphate insecticides inhibit the plasmatic cholinesterase enzyme (ChE) causing bradycardia, and inhibit the twitches of a nerve-muscle preparation, the insecticide effect of hydroalcoholic *N. exaltata* extract was evaluated through ChE determination and on neuromuscular twitches. The entire study was preceded by a quality control analysis of *N. exaltata* to authenticate either ashes or humidity content in its powder leaves.

## MATERIALS AND METHODS

### Plant

*Nephrolepis exaltata* specimens were commercially purchased from Kashima Flores®, box 30 of CEAGESP Sorocaba, SP, Brazil. The plant was initially identified by R.Y.H. Miura from Biological Sciences Course, Health Sciences Institute, Paulista University (UNIP), Sorocaba, SP, Brazil, and further confirmed by L. C. Bernacci from Instituto Agronômico de Campinas, where a voucher specimen was deposited (IAC number 57451).

### In vitro studies

Hydroalcoholic extract from leaves of *N. exaltata* and solubilization. The obtained vegetable drug mass was 938.7 g, when dried its yield was of 594.05 g (63.3% yield). An amount of 126.68 g of

leaves powder was taken and macerated along with 2.5 L of 70% ethanol. The obtained solvent was evaporated (Buchi rotavapor®, Valinhos, SP, Brazil) until dryness, and the dried extract (Thermo Fisher Scientific lyophilizer®, Australia) was then protected from light and humidity at room temperature until the assays.

### Quality control assays of the vegetal drugs – the ash and humidity tests

To observe their elementary physical and chemical characteristics, the powder obtained from the *N. exaltata* leaves was subjected to the ash and humidity tests (Brazilian Pharmacopoeia, 1998). Briefly, 100 g of powder specimen were placed in six calibrated melting pots, which were warmed until total powder carbonization. The melting pots were kept at 650°C and the ashes were weighed. Results were presented in grams of ashes/100 g of the sample. The humidity test was performed by placing 1g of powder specimen in each one of six calibrated porcelain capsules, which were warmed at 105°C for 4h and then weighed.

### Cholinesterase (ChE) inhibition assay

The extract was solubilized in polyethylene glycol 400 (PEG 400) (Sinth®, Diadema, SP, Brazil) according to Cintra-Francischinelli et al. (2008) to be used in the enzymatic reaction (ChE activity). The ability of the *N. exaltata* extract to inhibit ChE activity was assessed using a colorimetric assay (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil) that was standardized with a human serum calibrator of known ChE activity (BioControl N), according to the manufacturer's recommendations. BioControl N (Bioclin Quibasa), a pool of normal human serum, was used as internal quality control, according to the manufacturer's recommendations. The procedure was carried out according to Werner et al. (2015). The ChE activity was determined spectrophotometrically (UV-M51 spectrophotometer, BEL Engineering, Rio de Janeiro, RJ, Brazil) at 405 nm. The percentage of enzyme inhibition was calculated by comparing the enzymatic activity in the presence of 1.0 and 3.0 mg/mL of *N. exaltata* extract or with PEG 400 (20  $\mu$ L) used as solubilizer or with 1 mg/mL concentration of neostigmine (Sigma®) used as a cholinesterase inhibitor. The assays were done in triplicate.

### Preliminary toxicity

A preliminary toxicity assay without metabolization by the preincubation method (Maron and Ames, 1983) for future Ames test of *N. exaltata* extract was carried out using TA100 *S. typhimurium* strain (kindly provided by B.N. Ames, Berkeley, CA). The TA100 strain, a histidine dependent was selected as, all other *Salmonella* tester strains, and also contains a deletion mutation through the *uvrB*-bio genes, a mutation (*rfa*) that leads to a defective lipopolysaccharide (LPS) layer (Ames et al., 1973), and presence of plasmid pKM101 (Ames et al., 1975). In theory, mutagens can cause a base-pair substitution in TA100 as a reversion event. For a while, we measured the toxicity on TA 100 as a need to know enough concentration able to kill prokaryotic cells (Oliveira et al., 2019). The TA100 strain from frozen culture was grown overnight for 12 to 14 h in Oxoid Nutrient Broth No. 2. The recommended maximum concentration of 5 mg/plate (5,000  $\mu$ g/plate) *N. exaltata* extract (volumes of 25, 50, and 100 mL) were added to 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.4), with 0.1 mL of bacterial culture and then incubated at 37°C for 20 min. Next, 2 mL of top agar (0.6% agar, histidine and biotin 0.5 mM each, and 0.5% NaCl) was added and the mixture was poured into a plate containing minimal glucose agar (1.5% Bacto-Difco agar and 2% glucose in Vogel- Bonner medium). The plates were incubated at 37 °C for 48 h and the His+ revertant colonies were counted manually. All

experiments were performed in triplicate. For TA100, the standard mutagen used as a positive control in experiments without S9 mix was sodium azide (2.50 µg/plate). Dimethylsulfoxide (DMSO) was used as a negative (solvent) control (50 µL/plate). Toxicity was evaluated either as a reduction in the number of His<sup>+</sup> revertants or as an alteration in the auxotrophic background (Mortelmans and Zeiger, 2000; Yoshida et al., 2016).

## Ex vivo pharmacological experiments

### Animals

Eighteen male white Swiss mice (25-30 g) were purchased from Anilab (Laboratory Animals, Paulinia, SP, Brazil), and only 12 animals were used. The animals were housed at 25 ± 3°C (77 ± 3°F) in a light/dark cycle of 12 h and had access to food and water *ad libitum*. This study was approved by the Animal Ethics Committee of Sorocaba University (protocol n° 144/2019), and the experiments were carried out according to the international guideline - ARRIVE (Animal Research: Reporting of *In-Vivo* Experiments (Kilkenny et al., 2010).

### Mouse phrenic nerve-diaphragm muscle (PND) preparation

The diaphragm and its phrenic nerve branch were obtained from mice anesthetized with Halothane (Cristália®, Itapira, SP, Brazil) and sacrificed by exsanguination. Hemidiaphragms were mounted under a tension of 0.5 g in a 5 mL organ bath (Bülbring, 1997) containing Tyrode solution, and aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tyrode solution maintains the physiological conditions of the neuromuscular preparation at pH 7.0 and consists of (in mM): NaCl 137; KCl 2.7; CaCl<sub>2</sub> 1.8; MgCl<sub>2</sub> 0.49; NaH<sub>2</sub>PO<sub>4</sub> 0.42; NaHCO<sub>3</sub> 11.9 and Glucose 11.1. The preparation is indirectly stimulated through the phrenic nerve (ESF-15D double physiological stimulator), using supramaximal stimuli and a frequency of 0.06 Hz with a duration of 0.2 ms. Isometric twitch tension was recorded with a force-displacement transducer (cat. 7003, Ugo Basile, Italy) coupled to a digital recorder system (Data Capsule, cat. 17400, Ugo Basile) containing a Basic Pre-amplifier (cat. 7080, Ugo Basile), coupled to a computer via a USB interface for the data store. PND could stabilize for at least 20 min before the start of experiments. After recording under control conditions during 10 min stabilization of the preparation, the pharmacological protocols were performed using 2, 5, and 10 mg of PEG 400-solubilized *N. exaltata* extract added into the bath (n=6, each).

### In vivo study

#### *Allium cepa* test

This test has been widely used since it allows the evaluation of the effects or damages that mutagenic agents might cause. For the sample, it is necessary to be in constant mitotic division, seeking to identify the toxic effects and alterations during all cell cycle (Silva et al., 2018). Either the mitotic index as the replication index was used as indicators of adequate cell proliferation (Bonciu et al., 2018).

Briefly, scraped onion bulbs at the root (to promote the emergence of new roots) were exposed during 48 h for the root growth. They were divided into 5 groups for testing *N. exaltata* extract (at 2, 1 and 0.5 g solubilized in 1 L of water, n=6 each), positive control (paracetamol, 750 mg/L, n=6) (Bezerra et al., 2016), and negative control (potable tap water, n=6), in a total of 30 onion bulbs. After 48 h, the number of roots grown/bulb was counted and further removed and measured. Bulbs with minor root growth were

excluded from each group (Sumitha et al., 2016). The ends of major root grown were immersed in Carnoy's solution fixative (ethanol: acetic acid, 3:1), maintained at refrigerator for 24 h. Afterwards, two ends of each bulb were isolated and immersed in a heated HCl 1 N solution. Furthermore, they were transferred to microscopic slides and stained with toluidine solution 1.5 % and covered by a glass slip under slight pressure (squash technique). Quantification was made through optical microscopy in the objective of 10 or 40 giving increases of 100 or 400 X. A range of 50 - 85 cells per bulb to examine the presence of micronucleus.

### Data analysis

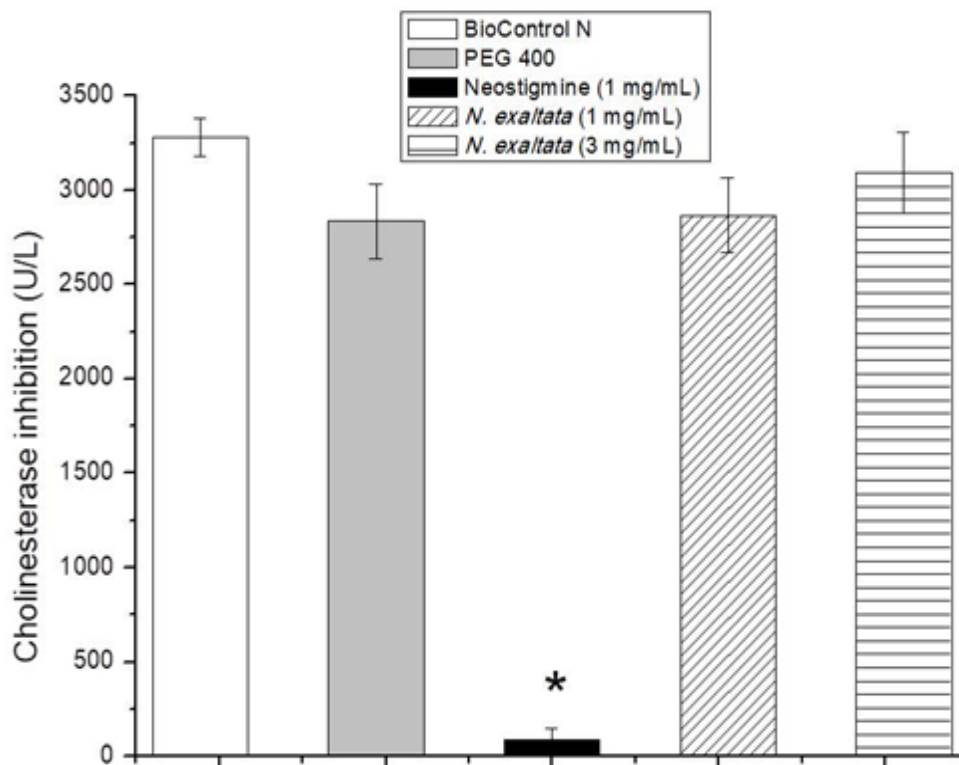
The data were expressed as the mean ± SEM (triplicate or n=6 depending on the experimental protocol). Results were analyzed by Student's *t*-test, with p<0.05 indicating significance. The *Salmonella*/Microsome results were analyzed using the Salanal statistical software package (U.S. Environmental Protection Agency, Monitoring Systems Laboratory, Las Vegas, NV, version 1.0, from Research Triangle Institute, RTP, North Carolina, USA), adopting the Bernstein et al. (1982) model. The data (revertants/plate) were assessed by analysis of variance (ANOVA) followed by linear regression. All data analyses were done using Origin® v.8.0 (OriginLab Corporation, Northampton, MA, USA).

## RESULTS AND DISCUSSION

*N. exaltata* extract was investigated on three approaches – a) *in vitro* (ashes and humidity; cholinesterase determination, and preliminary toxicity by *Salmonella*/Microsome); b) *ex vivo* (pharmacological profile on mammalian nerve-muscle synapse); and c) *in vivo* (genotoxicity using *Allium cepa* model), aiming to cover altogether pharmacognostic, biochemical, pharmacological and genotoxic parameters.

The elementary physical and chemical characteristics of *N. exaltata* such as ashes and humidity were carried out as important quality control of the plant, since excess water in herbal materials will encourage microbial growth, the presence of fungi, or insects, and deterioration following hydrolysis (Liu, 2019). Limits for water content should, therefore, be set for every given herbal material, being 11.7 ± 0.04 g/100 g in case of *N. exaltata* extract used here. On the other hand, ash values help determine the quality and purity of crude drugs in powder form, to remove all traces of organic matter resulting only inorganic residue (Pal et al., 2018), which measure is important, because mineral matter may be the cause of a pharmacological effect (Arraiza et al., 2017). *N. exaltata* extract showed 8.8 ± 0.11 g/100 g of ash content, like those found in leaves' powder of *Sesbania rostrata* (Momin and Kadam, 2011), an important dietary nutritive (amino acid, minerals, and antioxidants vitamins) source in countries of Southeast Asian.

Previous results obtained with *N. exaltata* were carried out using an aqueous solution from macerated leaves of *N. exaltata* fern, an ornamental plant considered to be safe (Popovici et al., 2018). When added to semi-isolated



**Figure 1.** Cholinesterase inhibition (U/L). The activity of *N. exaltata* extract at 1 and 3 mg/ml was compared with the known anticholinesterase agent neostigmine (1 mg/mL, positive control) and BioControl N (negative control). Notice that the plant extract has no inhibitory effect on cholinesterase enzyme activity.

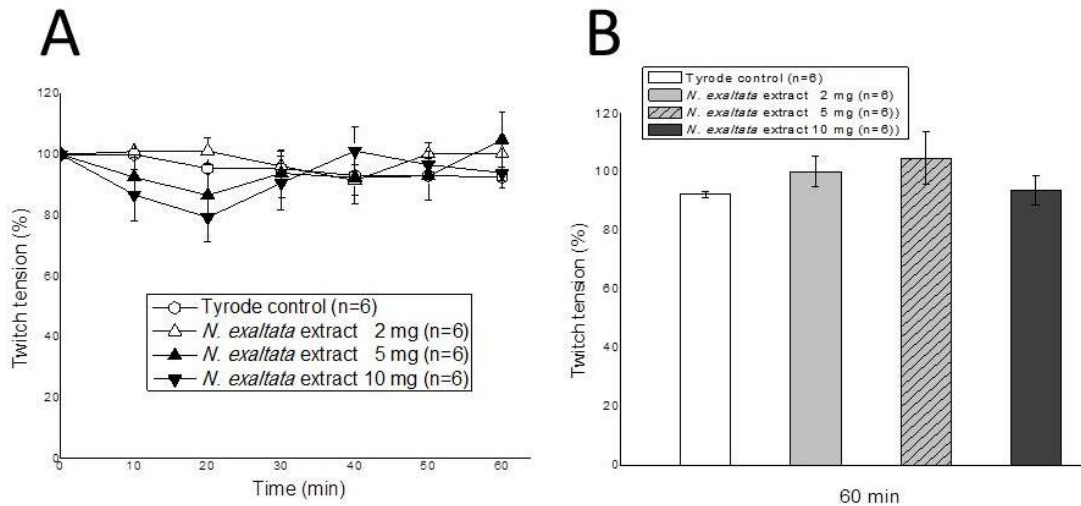
heart preparation of *Nauphoeta cinerea* the aqueous solution was cardiotoxic to cockroaches in a dose-dependent manner (Sanchez et al., 2018). The authors postulated an insecticide activity, as organophosphates are able since the plant exhibited an effect similar to that produced by acetylcholine (ACh) at the parasympathetic system.

Here, a validated protocol was used (Werner et al., 2015) to clear this question comparing the results with normal serum, negative control (BioControl N), and a known anticholinesterase agent, positive control, neostigmine (Table 1S). Figure 1 shows the contrasting results among negative control, the solubilizing PEG 400, and neostigmine. The mechanism by which aqueous *N. exaltata* inhibits the heartbeats was not caused by an anticholinesterase effect by the inhibition of the enzyme acetylcholinesterase. It is known that, in consequence of enzyme inhibition, the increased acetylcholine concentration at nerve terminals in areas other than the neuromuscular junction, several muscarinic receptor-mediated side-effects are seen, in the parasympathetic system, including abdominal cramps, diarrhea, increased gastric and bronchial secretions, salivation, lacrimation, nasal discharge, sweating, increased urination, and vagal effects as bradycardia (Kalla et al., 2016), prolonged QT

interval (Winter et al., 2018), and asystole (Nkemngu, 2017).

*Ex vivo* experiments using mouse phrenic nerve-diaphragm preparations are used to obtain the pharmacological effects of different compounds, a technique validated since 1946 by Büllbring (1997). The first effect showed by any anticholinesterase agent (acetylcholinesterase inhibitors) is a facilitatory effect showed by an increase of amplitude due to an increase of acetylcholine (ACh) release at synapses (Werner et al., 2015). Observing the Figure 2A none dose (2, 5 or 10 mg, n=6 each) of *N. exaltata* extract exhibited any facilitatory effect significantly different from Tyrode control. At the end of experiments at 60 min (Figure 2B), all concentrations were not different from control showing that the plant extract is not toxic to biological preparation since the basal response was maintained during the experiment.

Also investigated was the preliminary toxicity using *Salmonella*/microsome parameter, widely accepted short-term bacterial assay for identifying substances that can produce genetic damage that leads to gene mutations (Mortelmans and Zeiger, 2000), using a standard limit of 5 mg/plate (5,000 µg/plate), that is the maximum recommended dose for routine testing (Hamel et al.,



**Figure 2.** Mouse phrenic nerve-diaphragm preparation (indirect stimuli). Kinetic concentration-response curve of 2, 5, and 10 mg of *N. exaltata* extract (A) and at the end of the experiment (B), at 60 min. The number of experiments (n) is shown in the legend of the figure. No significant difference was observed when compared to Tyrode control ( $p > 0.05$ ).

2016). Table 2S shows the revertant colony counts after the *N. exaltata* extract exposure to TA100 (without metabolic activation, -S9) compared to negative and positive controls. Evaluation of toxicity is confirmed under the partial or complete absence of a background lawn or a substantial dose-related reduction in revertant colony counts, a fold response of less than 0.6-times compared to the negative control (Hamel et al., 2016). Figure 3 shows the absence of toxicity in *N. exaltata* extract visualized by colony counts in all tested concentrations (range of 625- 5,000  $\mu\text{g}/\text{plate}$ ). Preliminary toxicity is mandatory before the *Salmonella*/microsome achievement according to OECD Guideline for Testing of Chemicals, Bacterial Reverse Mutation Test (1997).

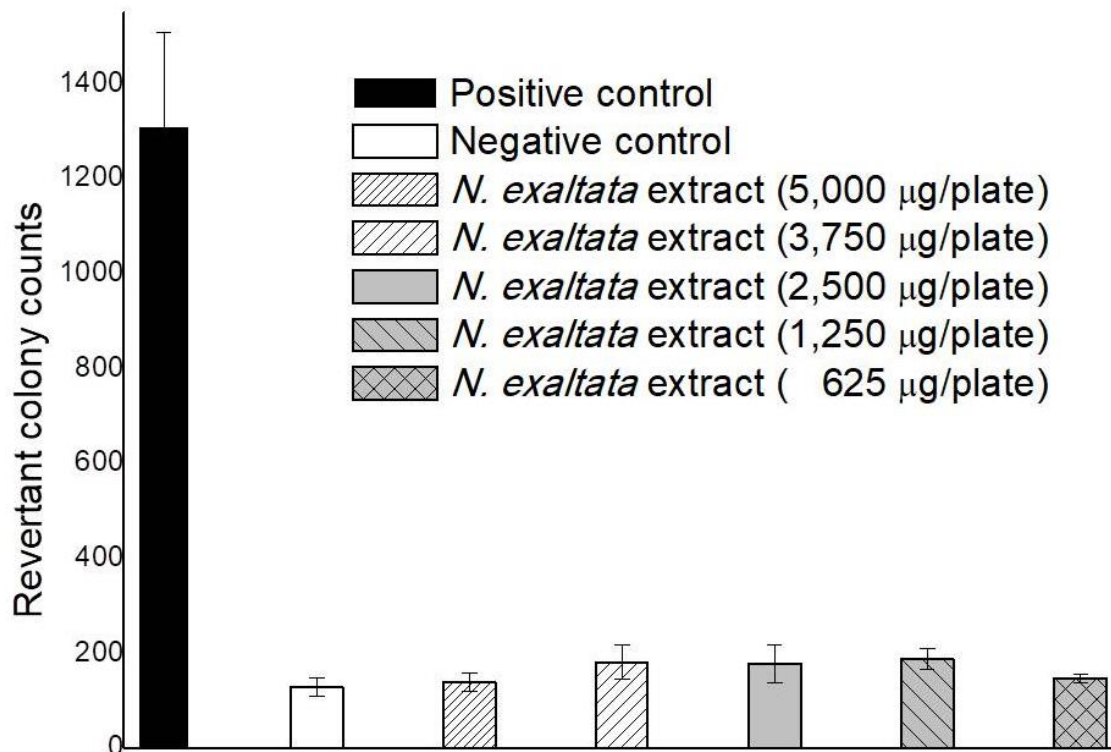
Tables 3S and 4S show the root growth and the altered cells count, respectively, of *N. exaltata* extract (2, 1 and 0.5 g), in comparison to negative (potable tap water) and positive (750 mg/L Paracetamol) controls. The major concentration of *N. exaltata* extract inhibited 100% of *A. cepa* root growth, being more effective as an inhibitor than the positive control paracetamol (Table 3S). Concerning the altered cell count (Table 4S) the presence of micronucleus in the selected areas was not observed neither in the positive control nor in *N. exaltata* extract. Figure 4 shows images of negative control, which in turn, did not differ from *N. exaltata* extract. Notice the absence of micronucleus in the selected parameter to visualize genotoxicity. In a study recorded by Informative Geum Bulletin (2015), the presence of micronucleus was found using 125 ( $0.16 \pm 0.05\%$ ), 250 ( $0.10 \pm 0.05\%$ ), and 500 ( $0.06 \pm 0.02\%$ )  $\mu\text{g}/\text{mL}$  paracetamol, although the values were not statistically different from negative control (distilled water;  $0.12 \pm 0.03\%$ ).

The *A. cepa* test allows the test of any compound (pure

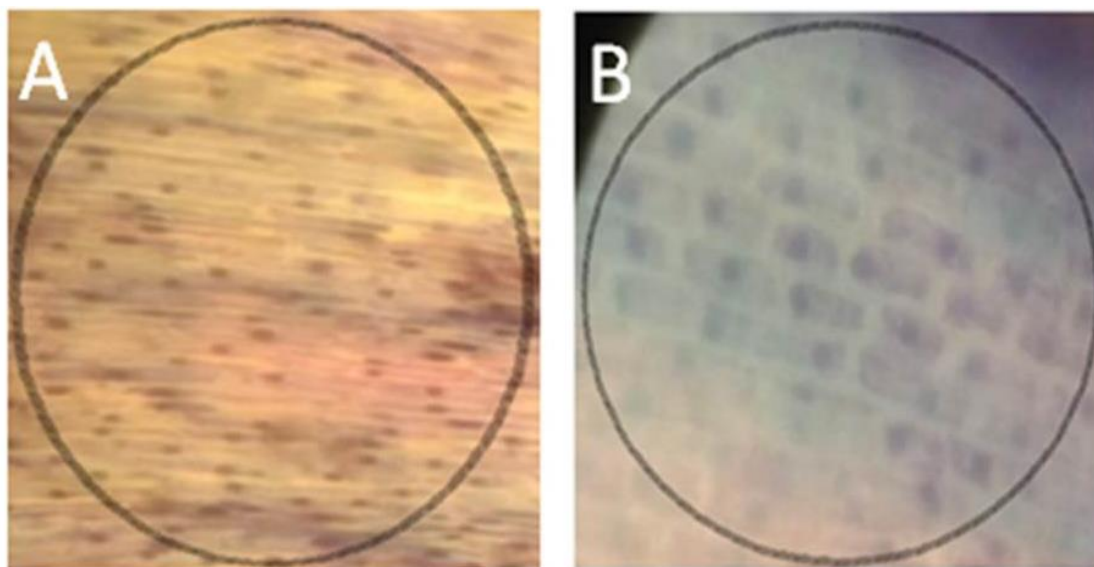
or in the mixture) able to cause damage to the DNA of eukaryotes (Bauer et al., 2015), and in an *in vivo* model, to secure safety a certain substance. The lack of genotoxicity in *N. exaltata* justifies its classification as a non-toxic plant even if aqueous extract causes the decrease dose-dependently the heartbeats of *Nauphoeta cinerea* (Sanchez et al., 2018). This pharmacological behavior and also the ability of *N. exaltata* extract in inhibiting the root growth of *A. cepa* would be explained by the presence of allelochemicals, as phenolics and terpenoids, in the plant. Allelochemicals are present in almost all plant tissues (leaves, flowers, fruits stems, roots, rhizomes, seeds, and pollen), and are released to the environment through volatilization, leaching, root exudation, and decomposition of plant residues (Sangeetha and Baskar, 2015).

The mechanisms by which allelochemicals act to inhibit germination, shoot, and root growth of other plants, involves the nutrient uptake destroying the plant's usable source of nutrients (Hassan et al., 2018), among others such as a generalized reduction in mitotic activity in roots and hypocotyls, hormone activity, rate of ion uptake, photosynthesis, respiration, protein formation, the permeability of cell membranes and/or enzyme action (Chang and Cheng, 2015). The presence of terpenes was identified, but not flavonoids, in *N. exaltata* aqueous extract (Sanchez et al., 2018), and it is known that terpenes inhibit the nitrification of soil, influencing the productivity of a plant community (Coskun et al., 2017). Corroborating these findings, the ethanol and aqueous extracts showed low cytotoxic effects on both *Triticum aestivum* roots and *Artemia franciscana* nauplii (Popovici et al., 2018). *N. exaltata* fern must be differentiated from bracken fern *Pteridium aquilinum* (L) Kuhn





**Figure 3.** Evaluation of toxicity (*Salmonella*/microsome assay). Revertant colony counts face to several concentrations of *N. exaltata* extract submitted to TA100 strain.



**Figure 4.** Characteristic photographs of negative control in two magnifications: A, 100 X; B, 400X. Micronucleus are absent in these images.

(Polypodiaceae) (Figure 2S), a specie that causes neoplasms of the urinary bladder and upper digestive tract related to the ingestion of bracken fern in cattle, due

to the presence of ptaquiloside (Agarwal et al., 2018) or in horses, by the toxic agent thiaminase (Reed et al., 2017).

## Conclusion

The plant has no anticholinesterase activity, is not toxic on bacterial reverse mutation or nerve-muscle synapses, neither on *A. cepa* assay, but inhibits the root growth of *A. cepa*, suggesting that *N. exaltata* does it as a mechanism of self-defense.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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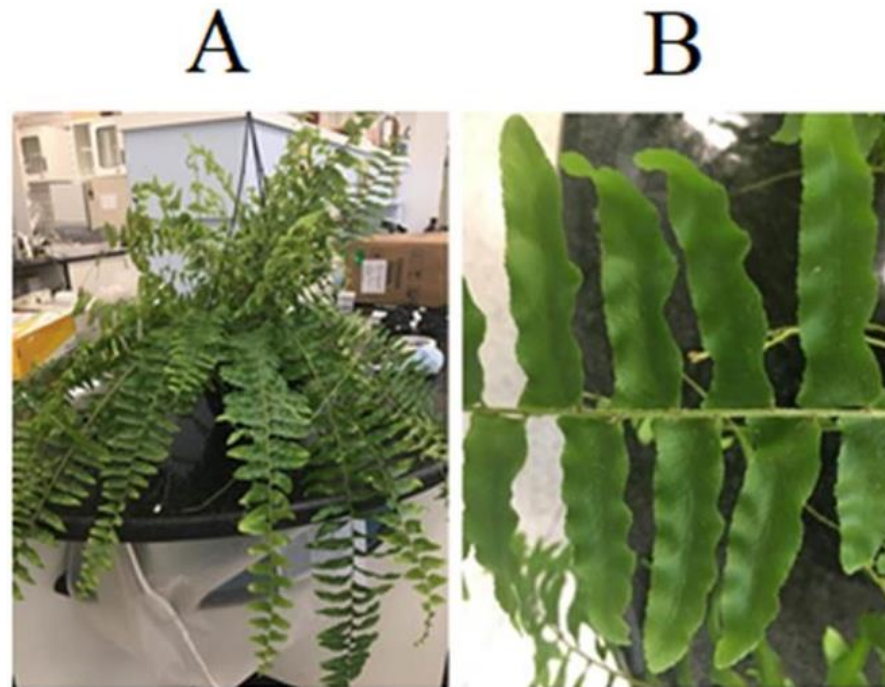
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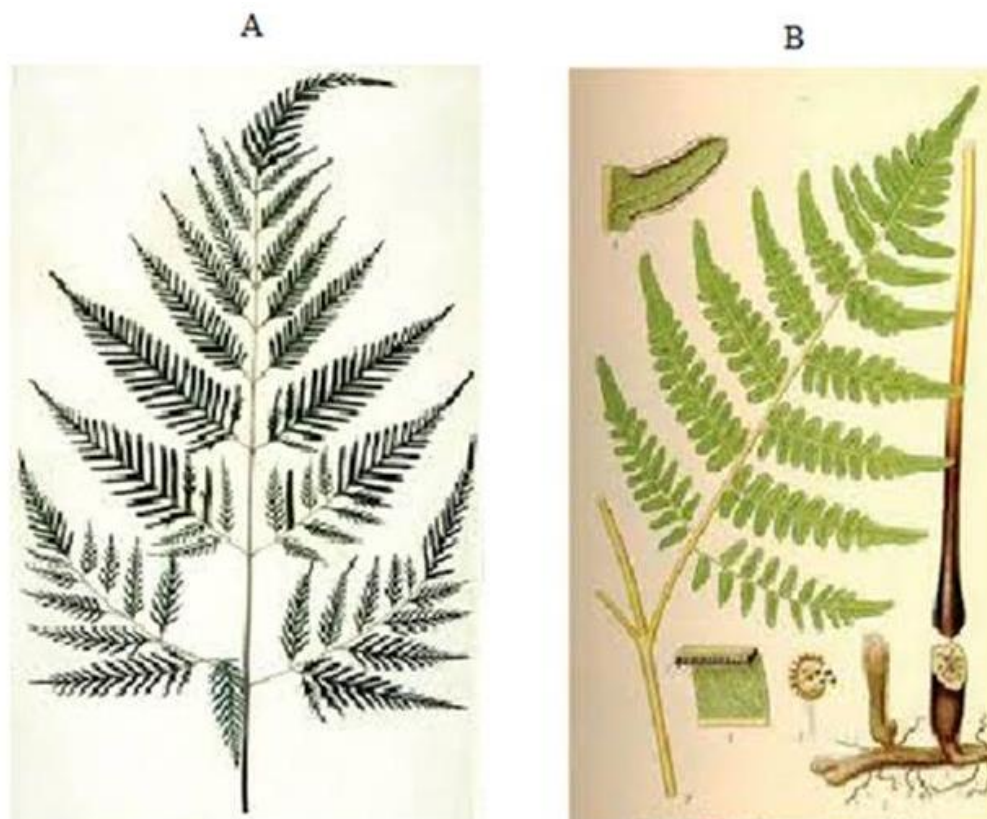
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**Figure 1S.** Ornamental *N. exaltata* (L.) Schott. (A) The appearance of a commercially obtained specimen. (B) Details of leaves.



**Figure 2S.** Images from the botanical illustration (A, pinterest.com) and exsiccate (B, figure uploaded by Elke Plessers) of bracken fern (*Pteridium aquilinum*), available at web in 20.10.2019.

**Table 1S.** Cholinesterase determination (in triplicate).

Treatment	Time (min)				ChE (U/L)	Mean $\pm$ S.E.M
	A0	A1	A2	A3		
BioControl N (Range: 2450 – 4000 U/L)	1778	1742	1706	1669	3166	3283.1 $\pm$ 100.6
	1761	1719	1684	1646	3341	
	1753	1716	1676	1638	3341	
PEG 400	1708	1687	1653	1618	2614	2837.6 $\pm$ 197.7
	1625	1594	1561	1525	2905	
	1730	1699	1663	1627	2992	
Neostigmine 1 mg/ml	1815	1816	1813	1810	145.2	87.1 $\pm$ 58.1 (*)
	1813	1817	1815	1812	29	
	1843	1846	1843	1840	87.1	
<i>N. exaltata</i> (1 mg/ml)	1766	1737	1704	1671	2687	2866.6 $\pm$ 200.7
	1700	1676	1638	1600	2828	
	1753	1718	1681	1644	3083	
<i>N. exaltata</i> (3 mg/ml)	1783	1748	1713	1665	3338	3092.9 $\pm$ 214.2
	1783	1748	1713	1679	2942	
	1784	1748	1713	1678	2998	

S.E.M., Standard Error Mean. \*,  $p < 0.05$  compared to BioControl N, PEG 400 and *N. exaltata*.

**Table 2S.** Preliminary toxicity of *N. exaltata* extract on TA100 strain (-S9) Salmonella/Microsome assay.

TA 100		N° of revertant/plate		
		$\mu\text{g/placa}$	N° revertant	$\pm\text{SD}$
<i>N. exaltata</i> (50 mg/ml)	C (+)	-	1310	197
	C (-)	-	130	19
	100 $\mu\text{l}$	5000	140	19
	75 $\mu\text{l}$	3750	182	36
	50 $\mu\text{l}$	2500	179	101
	25 $\mu\text{l}$	1250	188	22
	12.5 $\mu\text{l}$	625	149	9

**Table 3S.** Average root growth after exposure to different treatments.

Group	Average root growth (n=6) (cm)	Treatment	% of inhibition
Control -	1.4 $\pm$ 0.18	Potable tap water	0
Control +	0.7 $\pm$ 0.13	Paracetamol 750 mg/L	50
<i>N. exaltata</i> extract	0	2 g	100
	0.01 $\pm$ 0.03	1 g	93
	0.2 $\pm$ 0.18	0.5 g	85

**Table 4S.** Altered cells count.

Group	Total number of cells	Treatment	Altered cells
Control -	79	Potable tap Water	0
Control +	85	Paracetamol 750 mg/L	0
<i>N. exaltata</i> extract	None root growth	2 g	Not counted
	None root growth	1 g	Not counted
	57	0.5 g	0