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Research Article

Protection against the Phytotoxic Effect of Mercury Chloride by Catechin and Quercetin

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Plants when exposed to toxic levels of metals can suffer morphological or physiological damage because toxic metals can interact with several vital molecules in the plant. One possibility to remove these contaminants from the environment is through the phytoremediation technique, since secondary metabolites produced by plants can reverse these damages. To evaluate the cytoprotective activity, the dry mass and possible damage to the membranes of *Lactuca sativa* (lettuce) seedlings subjected to different concentrations of mercury chloride in association with catechin and quercetin in suballelopathic concentration were determined. The coordination of mercury chloride with substances was also evaluated using vibrational spectroscopy (Raman and FTIR). The interaction of the mentioned flavonoids with mercury chloride was evidenced through vibrational spectroscopy. When the metal was associated with catechin and quercetin, there was an increase in dry mass of almost 3 times when compared with the $HgCl_2$ alone, demonstrating that these flavonoids act as cytoprotective agents. However, in the presence of catechin and quercetin, membrane damage caused by mercury chloride has a level similar to that observed in control plants, demonstrating none statistical difference. Comparing the highest concentration with the lowest concentration of the metal associated with quercetin, it can be seen that the intensity of the peaks in this region decreases when the concentration of the metal increases, indicating an interaction between the metallic compound and the flavonoid. In this context, the use of secondary metabolites can be an alternative in the process of remediation of areas contaminated by mercury chloride, as they mitigate the effects of mercury chloride on lettuce seedlings.

1. Introduction

One of the most worrying issues today is the rise in pollution levels, whether atmospheric, aquatic, or terrestrial, which results in harmful conditions for organisms [1]. Among the main drivers of this scenario are toxic metals, responsible for the contamination and degradation of ecosystems [2].

Mercury is among the biggest environmental contaminants, causing significant reductions in biodiversity [3]. This metal with high toxicity content is usually found in inorganic form, such as mercury chloride ($HgCl_2$), and its disposal is related, for example, to the indiscriminate elimination of effluents from mining activities, the use of chemical products in agricultural practices, foundries, and industrial activities [4].

The use of natural products with antioxidant and chelating activities is among the main alternatives to reverse or prevent the toxic effects of metals on living beings [5]. Among these, phenolic compounds form one of the largest groups of secondary metabolites with therapeutic potential [6]. Furthermore, these compounds have high antioxidant activity, inhibiting the action of reactive oxygen species (ROS), thus avoiding lipid peroxidation that damages the cellular structure of organisms [7].

Flavonoids are among the most abundant phenolic compounds in vegetables, being concentrated in the aerial part of plants, such as flowers and fruits [8]. In addition to chelation of metals and protecting the body against oxidizing agents, these polyphenols are recognized for their bioactivities, such as anti-inflammatory, antitumor, antimicrobial, cardioprotective, and enzymatic inhibition [9].

Catechin and quercetin are flavonoids that, in addition to having the aforementioned biological activities, have gastroprotective, anticancer, antiallergic, antidiabetic, antihypertensive, vasodilatory, immunomodulatory, and antineurodegenerative effects [10–12]. In addition, they denote potential applicability in the remediation of areas contaminated by toxic metals, due to their significant antioxidant activity, free radical scavenging, and chelating mechanisms [13, 14].

Studies evaluating the interaction of phenolic compounds with toxic metals such as mercury are increasing, such as the studies by [15–17] and [18] aiming at their removal from contaminated environments. Thus, the present study was aimed at evaluating the cytoprotective effect of catechin and quercetin against the toxic action of mercury chloride on *Lactuca sativa* (lettuce) seeds, as well as vibration through interaction of compounds with spectroscopy (Raman).

2. Materials and Methods

2.1. Evaluation

2.1.1. Evaluation of the Cytoprotective Effect of Flavonoids in Lactuca Sativa against Mercury Chloride. To conduct the test, Petri dishes were prepared as described by Sobral-Souza et al. [19–21]. For the concentrations used, a suballelopathic concentration of the extract and fractions (8 μg/mL) and mercury chloride ($HgCl_2$) were used, ranging from 1.25 mM to 0.05 mM. The parameters analyzed at the end of seven days were as follows: germinated seed count, germination rate index (GRI), biometrics, and occurrence of root necrosis and seedling abnormalities, following the Seed Analysis Rules Manual [22]. The tests were done in triplicate and expressed as mean.

2.1.2. Electrolyte Leak Test. The percentage of damage to stem membranes and rootlets of germinated lettuce seeds was estimated using the electrolyte leak method described by Blum and Ebercon [23]. These plant tissues were separated and incubated in 25 mL of deionized water for a period of 2 hours at a temperature of 25°C. After that time, the electrical conductivity of the incubation solution was measured using an Oakton COM 700 conductivity meter. This first electrical conductivity reading was called L_1. Then, each tissue in its respective solution will be incubated in a water bath at 75°C for 30 minutes to release the cell electrolytes and cooled to room temperature, and the electrical conductivity of the solution will be determined as previously indicated. This second reading was called L_2. The following formula was used to calculate the percentage of membrane damage: $\mathrm{VE}\,(\%) = (L_2/L_1) \times 100$, where VE = percentage of membrane damage; L_1 = initial reading; and L_2 = final reading.

2.1.3. Fourier Transform Infrared Spectroscopy. For the analysis of the samples, an infrared absorption spectrometer by Fourrier FT-IR VERTEX 70 V, of the Bruker brand, was used. A Globar source is used for the medium infrared (MIR) region equipped with DL to TGS pyroelectric detectors to capture signals emitted from the sample. This equipment has a HeNe laser source with 633 nm of wavelength that allows the calibration of the optical path of the infrared beam next to the spectrometer mirrors. This spectrometer operates with a resolution of 2 cm^{-1} and a wide-range beam divider (beamspliter) composed of silicon that allows its use for measurements in the middle region. The use of vacuum in these experiments is important to improve the sensitivity of the detector.

2.1.4. Fourier Transform Raman Spectroscopy. The FT-Raman spectrum was recorded in the wavenumber range from 40 to 4000 cm^{-1} using a compacted powder of the sample in the sample holder of a Bruker RAM II FT-Raman module coupled to the VERTEX 70 spectrometer as well as a liquid nitrogen cooled high-sensitivity Ge detector. The samples were excited with the 1064 nm line of a Nd: YAG laser and we obtained a typical resolution of ~2 cm^{-1} with accumulation of 60 scans per spectra and a nominal laser power of 150 mW.

2.2. Statistical Analysis. All determinations were performed in triplicate and the results of chemical and vegetable tests were analyzed by calculating the arithmetic means with the Bonferroni and ANOVA.

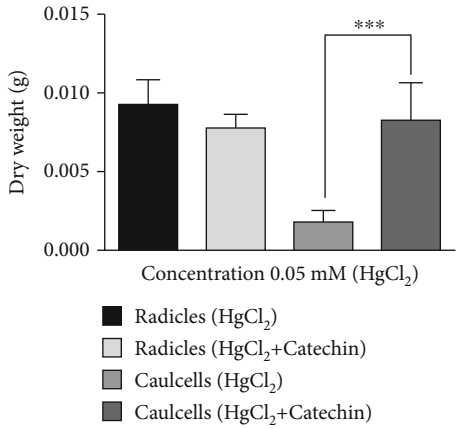

Figure 1: Effect of isolated mercury chloride ($HgCl_2$) and in association with catechin on the dry mass of radicles and stems of *Lactuca sativa*.

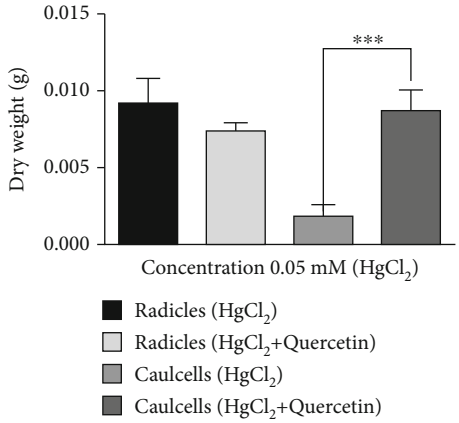

Figure 2: Effect of isolated mercury chloride ($HgCl_2$) and in association with quercetin on the dry mass of radicles and stems of *Lactuca sativa*.

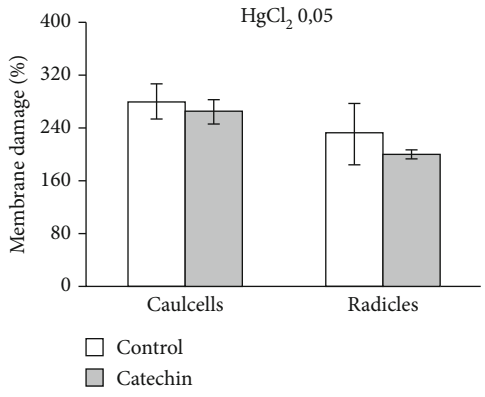

Figure 3: Membrane damage in *Lactuca sativa* caused by 0.05 mM $HgCl_2$ in the absence and presence of catechin.

3. Results

3.1. Growth of Stems and Roots. Figures 1 and 2 show the results of the action of mercury chloride on the dry mass of *L. sativa*. In the tested concentration of this metal, a reduction in the weight of the stems was observed, thus showing its interference in the development of plant tissues. However, when the metal was associated with catechin and quercetin, there was an increase in dry mass, demonstrating that these flavonoids act as cytoprotective agents, thus allowing for greater growth of the stems. When the action of mercury chloride alone or in association with the flavonoids used was evaluated, there was no interference in the dry mass of the radicles, thus showing that at a concentration of 0.05 mM, the metal did not alter the development of the plant species.

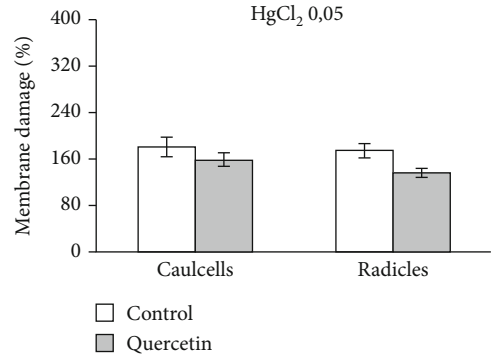

Figure 4: Membrane damage in *Lactuca sativa* caused by 0.05 mM $HgCl_2$ in the absence and presence of quercetin.

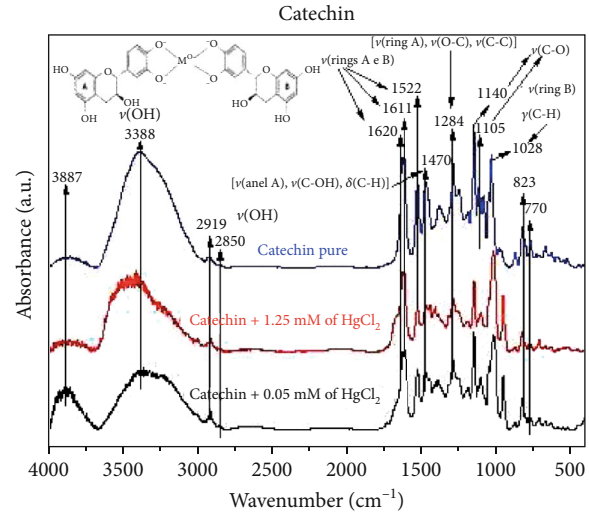

Figure 5: Spectra of pure catechin and associated mercury chloride at 0.05 mM and 1.25 mM.

3.2. Electrolyte Leaks. In this test, membrane damage in *L. sativa* caused by 0.05 mM mercury chloride in the absence and presence of catechin and quercetin was observed. Membrane damage was measured to observe the stability of *L. sativa* cell membranes as a function of mercury chloride concentrations when associated with these substances or alone. In this early stage of growth, the observation of damage to the membranes is essential and represents a good

Table 1: Characterization of the most relevant FTIR peaks of pure catechin and associated with mercury chloride.

Wavenumber (cm^{-1})			
Pure	0.05 mM $HgCl_2$	1.25 mM $HgCl_2$	Assignment
770	770	770	Deformation outside the OH group plane [24, 25]
823	823	823	δ (C-H) off plan [24, 25]
1028	1020	1020	Stretching of aromatic ring B and C-H bonds
1105	1103	1103	ν (C=O) [24, 25]
1140	1140	1140	ν (C=O) [24, 25]
1284	1284	1284	ν (ring A), ν (O-C), and ν (C-C) [24, 25]
1470	1466	1466	ν (anel A), ν (C-OH), and δ (C-H) [24, 25]
1522	1520	1520	ν (rings A and B) [24, 25]
1611	1611	1611	ν (rings A and B) [24, 25]
1620	1620	1620	ν (rings A and B) [24, 25]
2850	2850	2850	ν (O-H) [24, 25]
2919	2919	2919	ν (O-H) [24, 25]
3388			ν (O-H) [24, 25]
3887			

ν = stretching, δ = bending, r = rocking, and ω = wagging.

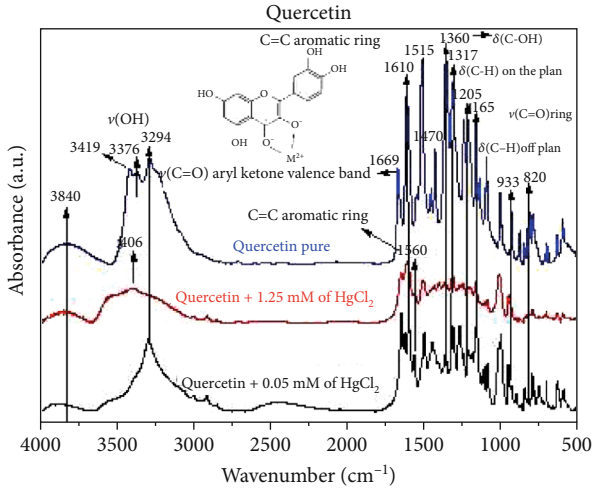

Figure 6: Spectra of pure quercetin and associated mercury chloride at 0.05 mM and 1.25 mM.

indicator to understand the toxic effect of the metal and the cytoprotective role of these flavonoids.

In Figures 3 and 4 the results show that in the presence of catechin and quercetin, membrane damage caused by mercury chloride has a level similar to that observed in the control plants (treated with water only). This preliminary result initially suggests this possible protective role of catechin and quercetin at a concentration of 0.05 mM against the toxicity of mercury chloride in both stems and radicles of *L. sativa*, this cytoprotection being statistically more relevant in the stems, which corroborates the electrolyte leakage tests.

3.3. FT Raman and FTIR Spectroscopy. The main bands characterized in the FTIR spectrum of the catechin, compared to the literature, were the stretching of the O-H bonds in the region of 3388 cm^{-1}, stretching of the C-H bonds in 2919 cm^{-1}, and aromatic ring stretch vibrations (A, B), also known as breathing vibration (Breathing). phenolic carbon-carbon bonds (1620, 1611, 1522 cm^{-1}). The peak in 1470 cm^{-1} was attributed to the stretch vibration of the connection of C-OH in phenol (A), while the peak 1284 cm^{-1} is associated with the aromatic ring A and the stretching of bonds COC and CC. The peak 1140 cm^{-1} was attributed to the stretching of the C-O link. The stretching of the aromatic ring B and C-H bonds was associated with the peak 1020 cm^{-1} [24, 25].

When observing the spectrum of pure catechin shown in Figure 5, a shift and decrease in peak intensity related to the O-H stretching occurs in the region of 3388 cm^{-1}, for the region of longest wavelength if we compare the smallest and the largest concentration with the metal.

There is a decrease in the peak intensities of 1522 cm^{-1} and 1470 cm^{-1} when catechin is put to react with $HgCl_2$ at concentrations of 0,05 and 1,25 mM. This decrease in peak intensity may be related to a possible coordination that the Hg^{2+} ion can make with the deprotonated oxygens of the phenolic groups present in the catechin structure. The decrease and small displacement of the peak in 1140 cm^{-1} also signals a possible coordination of the metallic ion with the catechin structure. A shift to lower energy frequencies is also observed in the stretch in 770 cm^{-1} referring to the out-of-plane deformation of the OH cluster (Table 1) [26].

Figure 6 shows the most relevant bands of quercetin. It is attributed to the OH stretch (Table 2) and the bands around 3400 cm^{-1}, the peak 1669 cm^{-1} is associated with the C=O vibration of the aryl ketone valence band. The peaks 1610, 1560 and 1515 cm^{-1} are associated with the C=C stretch vibration of aromatic rings. The spectrum also shows bending peaks from C-OH, in 1360 cm^{-1}, it is from OH in the phenol in 1377 cm^{-1}, in addition to CH flexion in the plane, in 1317 cm^{-1}. In 1205 cm^{-1} and 1165 cm^{-1}, we have peaks

Table 2: Characterization of the most relevant FTIR peaks of pure quercetin and associated with mercury chloride.

Wavenumber (cm^{-1})			
Pure	0.05 mM $HgCl_2$	1.25 mM $HgCl_2$	Assignment
820	820	820	δ (C-H) off plan [27, 28]
1165	1165	1165	ν (C=O) ring [27, 28]
1205	1205	1205	ν (C=O) ring [27, 28]
1317	1316	1316	δ (C-H) off plan [27, 28]
1360	1358	1357	δ (C-OH) [27, 28]
1515	1510	1510	C=C aromatic ring [27, 28]
1556	1560	1560	C=C aromatic ring [27, 28]
1610	1610	1510	C=C aromatic ring [27, 28]
1669	1654	1654	ν (C=O) aryl ketone valence band [27, 28]
3294	3294		ν (O-H) [27, 28]
3376			ν (O-H) [27, 28]
3406			ν (O-H) [27, 28]
3419			ν (O-H) [27, 28]

ν = stretching, δ = bending, r = rocking, and ω = wagging.

referring to stretch vibration C-O in the aromatic ring and bending out of plane in 820 cm^{-1} [27, 28].

It is observed by the infrared spectrum of pure quercetin and quercetin after the reaction with $HgCl_2$ at concentrations of 0,05 and 1,25 mM, a change in the band attributed to the carbonyl group which has a decrease in peak intensity in 1669 cm^{-1}. There is also a decrease in peak intensities in 1515 and 1360 cm^{-1}. This demonstrates a possible metal-to-ligand coordination from carbonyl oxygen.

The vibrational deformation originated from C-OH bonds is reflected in peaks formed in the region of 1300-1400 cm^{-1}, while the stretch deformations are located 1100 and 1200 cm^{-1} [28].

The peak reflected in the region between 3400 and 3200 cm^{-1} refers to symmetrical stretching O-H [29]. It is noticed that as there is an increase in the metal concentration, but mainly in the concentration of 1,25 mM, there is an absence of the peak absorbance. This could be due to the heavy metal binding that supposedly occurs in this radical.

4. Discussion

The results obtained about the effect of mercury chloride on the growth of *Lactuca sativa* and its dry mass are similar to those described by Rocha et al. [15–17] and Silva et al. [18], where they demonstrate the cytoprotective effect of several metabolites against the toxic action of mercury chloride.

Mercury at high levels affects seed germination and initial seedling growth, even though they have defense mechanisms against different types of stress, during germination and early development they become less tolerant [30–32]. Study with the translocation and biotoxicity of metal (oxide) nanoparticles in the wet plant system shows that *P. australis* are initially retained by the root system, followed by uptake and air transport throughout the plant [33].

Study carried out by Sobral-Souza et al. [34] showed that the presence of phenolic acids and flavonoids in samples of *Eugenia jambolana* demonstrated cytoprotective effect on the action of mercury chloride, in the stem and root development of lettuce seeds; in this study, the concentrations of mercury chloride were the same used in this research and the extract of this species also showed an expressive concentration of flavonoids in its composition.

Although the protection mechanisms using isolated substances are not fully clarified, it is preliminarily inferred that these substances may have reduced the absorption of mercury chloride by the lettuce roots or acted as an antioxidant system of the seedlings against the oxidative stress caused by this metal. Studies with the flavonoid rutin showed cytoprotection in lettuce seedlings, probably due to the antioxidant and chelating activity of this substance. Cytoprotection was verified in the eukaryotic plant model when rutin was associated with $HgCl_2$ at a concentration of 0.05 mM to promote the growth of radicles and stems, which corroborates these results [35].

Several studies have documented adverse effects caused by toxic metals in plants, such as changes in the pattern of germination and growth and morphology of radicles, stems, and leaves [36, 37]. Studies using the plant supercritical carbon dioxide (SFE) system to evaluate flavonols that play a key role in heavy metal resistance have indicated that flavonoids can improve resistance to oxidative stress by quenching the ROS [38].

[26] studying the coordination of the gallium ion, the structure of quercetin proposed that the hydroxyl group of the carbon neighboring the carbonyl has a more acidic proton, when compared to the other protons of the other phenolic groups. This fact results in a greater possibility of coordination of the metal ion occurring via carbonyl oxygen and oxygen from the deprotonated phenolic group. Probably the other phenolics should not be involved in the coordination of the metallic ion due to the lower acidity and the possible spatial impediment caused by the first complexation.

Flavonoid compounds have the ability to form complexes with metallic ions and exhibit the greatest

physiological strength, such as the association of quercetin [39]. Analyzing the infrared spectrum of isolated quercetin, it can be noted that the peaks related to the vibration of the aromatic ring are concentrated in the region of 1400 and 1600 cm^{-1}. Comparing the highest concentration with the lowest concentration of the metal associated with quercetin, it can be seen that the intensity of the peaks in this region decreases when the concentration of the metal increases, indicating an interaction between the metallic compound and the flavonoid [28, 40].

Previous studies such as the one by [41] had already demonstrated the complexation capacity of flavonoids, especially rutin, which had greater activity in the scavenging of superoxide radicals when complexed to copper and iron metals.

5. Conclusions

This study suggests that although mercury chloride causes cytotoxicity in several plant species, its complex with the flavonoids catechin and quercetin can minimize these effects, thus allowing for better plant growth, which can be observed by preserving plant tissue, *Lactuca sativa*, demonstrated by the increase in dry mass. Although these results are innovative and promising regarding the search for new alternatives for the decontamination of areas polluted by toxic metals, further studies are needed to observe the effect of their application in contaminated areas.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

S.R.T., J.C.A.P., C.E.S.S., A.K.S., J.C.A.P., J.H.d.S., and H.D.M.C. were responsible for the conceptualization; Y.M.L.S.d.M. and J.B.d.A.N. were responsible for the methodology; A.K.S. was responsible for the software; D.L.M.V., A.C.H.B., F.F.C., and M.M.C.d.S. were responsible for the validation ; Y.M.L.S.d.M. was responsible for the formal analysis ; Y.M.L.S.d.M. and J.B.d.A.N. were responsible for the investigation; H.D.M.C., T.T.A.Y., A.S., and R.N.P.T. were responsible for the resources; A.K.S. was responsible for the data curation; Y.M.L.S.d.M. and J.B.d.A.N. were responsible for writing and original draft preparation; J.E.R., C.E.S.S., and B.K. were responsible for writing, reviewing, and editing; J.B.d.A.N. and M.M.C.d.S. were responsible for visualization; H.D.M.C. and J.C.A.P. were responsible for the supervision; H.D.M.C. was responsible for the project administration; A.S. and B.K were responsible for the funding acquisition. All authors have read and agreed to the published version of the manuscript.

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