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USE OF BIOASSAY WITH *Allium cepa* L. FOR THE CYTOTOXICITY ASSESSMENT OF *Croton urucurana* Baill

USO DE BIOENSAIO COM *Allium cepa* L. PARA AVALIACÃO DE CITOTOXICIDADE DE *Croton urucurana* Baill

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RESUMO: *Croton urucurana* Baill é uma planta medicinal conhecida popularmente como Sangra d'água, pois quando seu tronco é cortado, libera uma seiva resinosa de cor vermelha escura como sangue. A casca e a resina dessa planta são utilizadas como remédio natural por suas propriedades de anti-inflamatórias, antibacterianas, antissépticas e cicatrizantes. Embora, há poucos estudos que comprovam sua eficácia e segurança. O uso de ensaios biológicos para avaliar a bioatividade dos extratos e compostos de plantas foram frequentemente aplicados para identificação e monitoramento de substâncias potencialmente tóxicas. Este estudo tem como objetivo avaliar a citotoxicidade do extrato de resina de planta medicinal *C. urucurana* por meio de bioensaio com *Allium cepa* (cebola). As cebolas descascadas foram colocadas em recipiente com água destilada durante 24 horas para estimular crescimentos das raízes. Depois desse período, foram escolhidos bulbos de cebolas saudáveis para o experimento e tratados com seIs diferentes concentrações de resina (25, 50, 100, 250, 500 e 750 ppm). O ensaio foi realizado em 96 horas. As cebolas tratadas com água destilada foram utilizadas como controle. Ao final do ensaio foi mensurado o tamanho das raízes de bulbos, dez amostras para cada tratamento. Os resultados mostraram que os extratos nas doses e tempos de exposição avaliado, foram citotóxico e retardaram os crescimentos radiculares das cebolas. O comprimento das raízes diminuiu com o aumento das concentrações do extrato. As médias do grupo de tratamentos são significativamente diferentes da média do controle (p<0,05), analisados pelo teste de *Dunnett's*. As taxas de inibição foram superiores a 50%

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a 250, 500 e 750 ppm com valores 63, 81 e 86%, respectivamente. Em resumo, a resina causam uma inibição do crescimento radicular do *Allium* cuja intensidade depende das concentrações aplicadas.

Palavras-chave: *Allium cepa* L. Citotoxicidade. *Croton urucurana* Baill

ABSTRACT: *Croton urucurana* Baill is a medicinal plant popularly known as *Sangra d'água* because when its trunk is cut, it produces resin and is a dark red color like a blood. The bark and the resin of this plant are used as a natural remedy due to anti-inflammatory, antibacterial, antiseptic, and healing properties. However, there are few studies that prove its effectiveness and safety. The use of biological assays to assess the bioactivity of a plant extract and compound has often been applied for the identification and monitoring of potentially toxic substances. This study aims to evaluate the cytotoxicity of the resin extract of medicinal plant *C. urucurana* through bioassay with *Allium cepa* (onion). The peeled onions were placed in a container with distilled water for 24 hours to stimulate the root growth. After that period, the healthy onion bulbs were selected for the experiment and were treated with six different resin concentrations (25, 50, 100, 250, 500, and 750 ppm). The test was carried out for 96 hours. The onions treated with distilled water were used as its control. At the end of the trial, the root size of the bulbs was measured, were selected ten samples for each treatment. The results showed that the extracts in the doses and exposure times evaluated, were cytotoxic and delayed the roots growth of the onions. The roots length decreased with increasing the extract concentrations. The means of treatments group are significantly different from the mean of the control ($p<0.05$), analyzed using Dunnett's test. The inhibition rates were more than 50% at 250, 500, and 750 ppm with values 63, 81, and 86% respectively. In summary, the resin caused an inhibition in the *Allium* root growth which the intensity depends on the concentrations applied.

Keyword: *Allium cepa* L. Cytotoxicity. Croton urucurana Baill.

INTRODUCTION

According to the National Health Surveillance Agency (ANVISA, s/d), medicinal plants are several types of plants capable of relieving or curing diseases and apply as a traditional or popular medicine in community. In 1991, the World Health Organization (WHO) reinforced the important contribution of popular medicine in providing social assistance, especially to the communities that have little access to health systems and asked member of the states to intensify the cooperation between traditional medicine practitioners and modern health care (BRASIL, 2006). According to WHO, traditional medicine and medical practices that refer to medicine as complementary or alternative, have been increasingly used in both developed and developing countries, and recognized that 80% of populations in developing countries use traditional medicine to care for their Cheers.

Croton urucurana Baill of the *Euphorbiaceae* family is a medicinal plant widely found in the Midwest region of Brazil. Popularly known as *Sangra d'água*, when an injury occurs at the bark of a tree, it produces resin and is dark red color like a blood. The bark and the resin of this plant are used in popular tradition as a natural remedy for their anti-inflammatory, antibacterial,

antiseptic, and healing properties (LORENZI; MATOS, 2008). Although, there are few studies that prove its effectiveness and safety. The genus *Croton* is one of the most numerous in the *Euphorbiaceae* family, presenting a total of 700 to 800 species and about 300 of them have been cataloged in Brazil (SILVA, 1999). The main chemical components of *Croton* are tannins, lignans and taspine (alkaloid). According to Vaisberg (1989), *Croton lechleri* taspine has anti-inflammatory and antioxidant properties and acts as a potent acetylcholinesterase inhibitor and healing agent. Silva (1999) stated that the resin of *C. urucurana* is highly toxic orally by testing in Wistar rats. Another species, *Croton sp.* was used to treat lung cancer (HUBER, 2010).

Toxicity is defined as a property inherent in the substance that produces harmful effects to exposed organisms for a certain time that produces adverse effects such as: inhibition of reproduction and growth of the tested organism or mortality and immobility (ARRAES; LONGHIN, 2012). The use of biological tests to evaluate bioactivity of plant extracts and compounds has often been applied for the identification and monitoring of potentially toxic substances (IGANCI, 2006). According to Grant (1999), the bioassay with plants has been considered quite sensitive and simple in monitoring the cytotoxic effects (toxic in living cells) of chemical compounds. *Allium cepa* L. has been indicated as an efficient test for the evaluation of cytotoxicity due to its properties of kinetic proliferation (GOMES et al., 2013). Its reduced number of large chromosomes (2n = 16) and changes in cells facilitate the observation of results resulting from the action of chemical compounds (RODRIGUES, et al., 2016). This work aims to evaluate the cytotoxicity of the resins extract of medicinal plant *Croton urucurana* Baill through a bioassay with *Allium cepa* L.

MATERIAL AND METHODS

Material

The resins of *C. urucurana* was collected at the site near the campus of Instituto Federal de Goiás (IFG) Aparecida de Goiânia and in rural area of the municipalities of Nova Fatima and Hidrolandia in Goiás. The plant species was identified by the Botany Department of the Federal University of Goiás (UFG) and the exsiccate was deposited in the herbarium with the registration number 50320. The resin (Figure 1a) was collected by incisions made in the bark of the plant's stem, using a sharp blade. The resin was kept inside closed bottles and stored in the freezer. To carry out the study, equal-size *Allium cepa* (onions) were selected and purchased from the local store. The onions were kept cold and dry until the experiment.

Bioassay

The onions were carefully peeled, and the brown tunic was removed without destroying the primordial root. Figure 1b, clean, peeled onions were placed in a container with distilled water for 24 hours to stimulate the root growth (OZKARA et al., 2015; GOMES et al., 2013). After 24 hours, we selected the healthy onion bulbs used for the experiment. The roots were treated with seven different concentrations of resin at: 25 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 750 ppm. The test was carried out in 96 hours at room temperature (OZKARA, 2015). After 96 hours of exposure of extract, the roots lengths were measured and used as an index of toxicity. Onions treated with distilled water were used as the group control.

Statistical analysis

The mean ± SD (standard deviation of the root length for the treatments group and the control were calculated. The data were analyzed statistically by measuring SD and one-way analysis of variance (ANOVA) and pos hoc Dunnett's test. The test was performed to compare the significant differences between the treatments group to a single control group at p value less than 0.05.

a)

b)

Figure 1: a) Resins produced by *C. urucurana* Baill b) Equal-size bulbs *Allium cepa* without any treatment were placed in a container with distilled water for 24 hours to stimulate the root growth.

RESULTS AND DISCUSSION

After 96 hours (four days) of experiment, the mean length of root for the control group was 4,29 ± 0.39 cm. The results (Table 1) also showed that the roots growth decreased with increasing resin concentrations (25, 50, 100, 250, 500, and 750 ppm). The average length of the root was 2,92 ± 0.66 cm when treated with 25 ppm resin extract and gradually decreased until reached 0.62 ± 0.17 cm at 750 ppm concentration. All treatments group means are significantly different from the control group mean ($p < 0.05$), done by Dunnett's test. The *C. urucurana* were found to be cytotoxic and retarded the root growth of *Allium cepa* in the doses and exposure times

evaluated. The inhibitions were 32% at 25 ppm concentration then increased to 38% at 50 ppm and 39% at 100 ppm compared to the control group. The effective concentrations reduced the root growth more than 50% at 250, 500, and 750 ppm with inhibition rates of 63, 81, and 86% respectively.

Table 1: *Allium cepa* root growth inhibition test for 96 hours

Test substance	Concentration (ppm)	Root length (cm) Mean± SD	Inhibition (%)
Control	Distilled water	4.29 ± 0.39	-
C. urucurana	25	2.92 ± 0.66	32
	50	2.64 ± 0.58	38
	100	2.61 ± 0.70	39
	250	1.57 ± 0.31	63
	500	0.80 ± 0.13	81
	750	0.62 ± 0.17	86

The mean length of the root for all treatments were significantly different from the mean of the control. SD: Standard deviation.

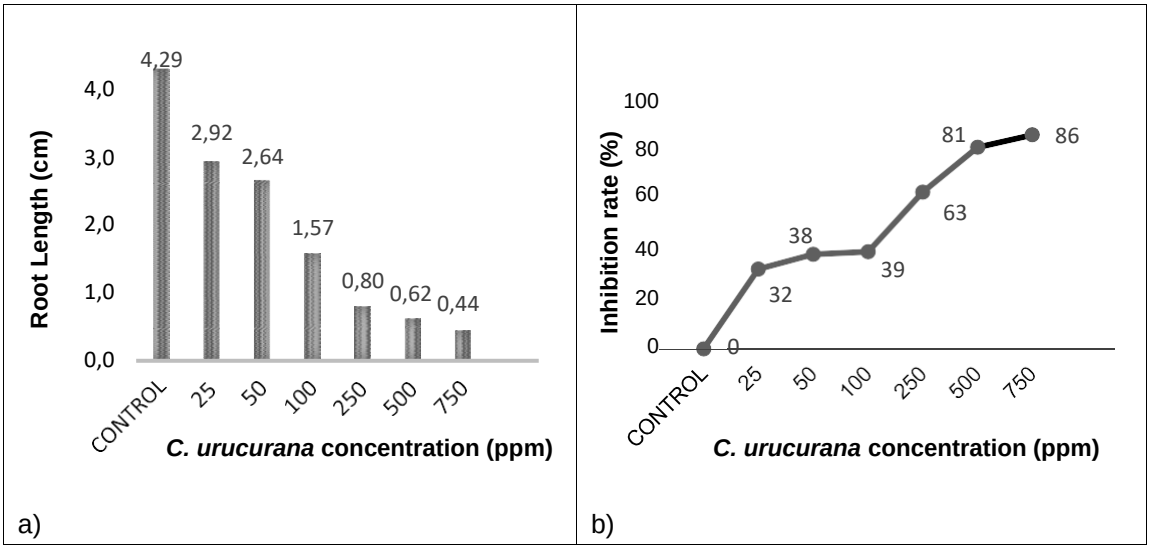

Figure 2: a) The effects of *C. urucurana* resin on the growth of *Allium* roots. b) The percentages of the inhibition rates increased significantly at 250 ppm concentration.

According to Candido et al. (2013), the global process of plant germination consists of three partial processes: imbibition, activation and intraseminal growth. The changes in the germination prototypes with the influence of the inhibitor such as *C. urucurana* resin enable results of different effects at the primary level. Among others, it shows if changes in the permeability of membranes, in the transcription and translation of DNA, in the action of secondary messengers, in breathing by the sequestration of oxygen, in the formation of enzymes

and receptors, or even, by the combination of these factors (FERREIRA & AQUILA, 2000).

Allium cepa is one of the most effective plant used in bioassay for the determination cytotoxic and genotoxicity effects due to its sensitivity (BARBERIO et el., 2011). Table 1 shows the negative effects on the root growth of *Allium* in the presence of *C. urucurana* extract. The most accentuated inhibitory effects were determined at the concentrations 250 ppm or greater, with the inhibition percentage more than 50%. It was observed as the resin concentrations level increased, the inhibition rates increased, and the roots length decreased significantly (Figure 2).

The interference of the plant growth in the presence of the inhibitor is associated with a strong inhibition of mitosis (CANDIDO, 2013). This fact can be attributed to the ability of the compounds present in the inhibitor to reduce the mitotic index, partially blocking the subsequent phases of cell division in the highest concentrations. GNIAZDOWSKA and BOGATEK (2005) also pointed out that the reduction of the plant growth in the presence of allelochemicals is associated with a strong inhibition of mitosis and/or disruption of the structure of organelles, such as nucleus and mitochondria.

Similar results were confirmed by Simionatto et al. (2009) under phytotoxic effect on the germination and growth of lettuce and onion, with 100% inhibition in the growth of lettuce and onion, compared to the control, when subjected to the volatile oil of the stem of *C. urucurana*.

CONCLUSION

In the present experiment it can be concluded that the resin extract of *C. urucurana* was found to be cytotoxic in *Allium* test. The level of the cytotoxicity activities depends on the doses applied. It suggested that the use of resin *C. urucurana* as natural remedy can damage the cell when use in high doses and improperly.

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