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Evaluation of antioxidant activity and toxicity in *Artemia salina* of the ether extract and fractions from *Tecoma stans* seeds

Avaliação da atividade antioxidante e toxicidade em *Artemia salina* do extrato etéreo e frações de sementes de *Tecoma stans*

Evaluación de la actividad antioxidante y la toxicidad en *Artemia salina* a partir de extracto etéreo y fracciones de semillas de *Tecoma stans*

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Abstract
Tecoma stans (L.) Juss. Ex Kunth is a species belonging to the Bignoniaceae family, popularly known as yellowing, yellow bell, and garden little ipe. Studies with *T. stans* seeds are scarce and it have potential in the search for natural compounds with biological activities. This study aimed to evaluate the toxicity and antioxidant activity of the ether extract (EE) and fractions from *T. stans* seeds. The EE was obtained in Soxhlet apparatus with petroleum ether and fractions were obtained by hydrolysis and esterification reactions. Phytochemical screening evaluated the presence of steroids, triterpenoids, alkaloids, coumarins and anthraquinones in EE. The toxicity was evaluated by the *A. salina* lethality test and antioxidant activity by DPPH method. It was observed the presence of steroids, triterpenoids and alkaloids in EE, which had no toxicity to *A. salina* (LC_{50} > 1000 µg/mL). The fractions of seeds exhibited toxicity on *A. salina.* All samples showed antioxidant activity with EC_{50} between 0.9 and 1.1 µg/mL. These results indicated potential applications for *T. stans* seeds.
Keywords: *Tecoma stans*; Secondary metabolites; Antioxidant activity; Brine shrimp.

Resumo
Tecoma stans (L.) Juss. Ex Kunth é uma espécie pertencente à família Bignoniaceae, popularmente conhecida como amarelinho, sino amarelo e ipezinho de jardim. Estudos com sementes de *T. stans* são escassos e tem potencial na busca de compostos naturais com atividades biológicas. Este trabalho teve como objetivo avaliar a toxicidade e a atividade antioxidante do extrato etéreo (EE) e frações de sementes de *T. stans*. EE foi obtido em aparelho de Soxhlet com éter de petróleo e as frações foram obtidas por reações de hidrólise e esterificação. A triagem fitoquímica avaliou a presença de esteroides, triterpenoides, alcaloides, cumarinas e antraquinonas no EE. A toxicidade foi avaliada pelo teste de letalidade em *A. salina* e a atividade antioxidante pelo método de DPPH. Foi observada a presença de esteroides, triterpenoides e alcaloides no EE, que não apresentou toxicidade para *A. salina* (CL_{50} > 1000 µg/mL). As frações do EE das sementes de *T. stans* exibiram toxicidade em *A. salina*. Todas as amostras apresentaram atividade antioxidante com CE_{50} entre 0,9 e 1,1 µg/mL. Esses resultados indicam potenciais aplicações para sementes de *T. stans*.
Palavras-chave: *Tecoma stans*; Metabólitos secundários; Atividade antioxidante; *Artemia salina*.

Resumen
Tecoma stans (L.) Juss. Ex Kunth es una especie perteneciente a la familia Bignoniaceae, popularmente conocida como amarillamiento, campana amarilla y ipezito de jardín. Los estudios con semillas de *T. stans* son escasos y tienen

potencial en la búsqueda de compuestos naturales con actividades biológicas. Este trabajo tuvo como objetivo evaluar la toxicidad y actividad antioxidante del extracto etéreo (EE) y fracciones de semilla de *T. stans*. El EE se obtuvo en un aparato Soxhlet con éter de petróleo y las fracciones se obtuvieron mediante reacciones de hidrólisis y esterificación. El cribado fitoquímico evaluó la presencia de esteroides, triterpenoides, alcaloides, cumarinas y antraquinonas en EE. La toxicidad se evaluó mediante la prueba de letalidad en *A. salina* y la actividad antioxidante mediante el método DPPH. En el EE se observó la presencia de esteroides, triterpenoides y alcaloides, los cuales no mostraron toxicidad para *A. salina* (CL_{50} > 1000 µg/mL). Las fracciones de EE de las semillas de *T. stans* mostraron toxicidad en *A. salina*. Todas las muestras mostraron actividad antioxidante con CE_{50} entre 0.9 y 1.1 µg/mL. Estos resultados indicaron aplicaciones potenciales para las semillas de *T. stans.*
Palabras clave: *Tecoma stans*; Metabolitos secundarios; Actividad antioxidante; Camarón de salmuera.

1. Introduction

Tecoma stans (L.) Juss. Ex Kunth is a species belonging to the Bignoniaceae family, popularly known as yellowing, yellow bell and garden little ipe (Kranz & Passini, 1997). This species exhibits antibacterial, antioxidant, antinociceptive, anti-inflammatory, antidiabetic and larvicidal activities; and these effects are correlated to presence of alkaloids, anthraquinones, phenolic compounds, steroids, glycosides, hydrocarbons, essential oils, tannins, terpenes and saponins (Alonso-Castro et al., 2010; Prasanna et al., 2013; Salem et al., 2013). Studies with *T. stans* seeds are scarce and it have potential in the search for natural compounds with biological activities.

An important problem that concerns researchers are diseases caused by the uncontrolled production of free radicals. Many diseases, such as cardiovascular diseases, cancer, Alzheimer's and cataracts, are correlated to excess free radicals in the body (Neha et al., 2019; Pohanka, 2018). Various plants have polyunsaturated fatty acids (omega 3 and omega 6) and carotenoids, which may be applicable in the treatment of diseases associated with oxidative stress, that promote lipid peroxidation, damage to DNA, enzymes and proteins (Martin et al., 2006; Simopoulos, 2002).

Due to the scarcity of studies that prove the biological potential of plants used by the population, it is necessary to evaluation for the toxicological activity of these extracts to ensure their safe use. Tests with *A. salina* allow the assessment with sensitivity, being an indicative model of toxicity for animal species that make up the ecosystem (Favilla et al., 2006).

Thus, the objective of this work was to evaluate the antioxidant activity and toxicity of the ether extract and fractions from *T. stans* seeds.

2. Methodology

This work is an experimental research with a quantitative approach (Pereira et al., 2018), carried by data collection through the use of measurements of values, being the application of this method necessary to verify the results obtained from the objectives proposed.

2.1 Chemicals

Petroleum ether, methanol (CH_3OH), potassium hydroxide (KOH), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), anhydrous sodium sulfate (Na_2SO_4) and hexane (C_6H_{14}) were purchased from Dinâmica® (Brazil); 2,2-diphenyl-1-picryl-hydrazine (DPPH) and dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich® (Germany); 2,6-di-tert-butyl-4-methyl phenol (BHT) was purchased from Merck® (Germany); *Artemia salina* eggs and synthetic sea salt were purchased from Maramar® (Brazil) and Marinemix® (Brazil), respectively.

2.2 Plant material

The plant material was collected in a Cerrado area located in Divinópolis, Minas Gerais State (20º10’44’’S latitude and longitude 44º55’6” W GRW). The vouchers were identified as *Tecoma stans* (L) Juss. Ex Kunth by Andréia Fonseca Silva

and deposited in the PAMG Herbarium, belonging to the Minas Gerais Agricultural Research Corporation (EPAMIG), under registration number 58284. This study has access permission to the components of plant genetic heritage and it is registered in the SisGen Platform (Register AEF6C95), according to Brazilian Biodiversity Law (13.123/2015).

2.3 Obtaining extract and fractions

To obtain ether extract (EE), the plant material was exhaustively extracted with petroleum ether at 40 °C in the Soxhlet apparatus for 40 cycles. For hydrolyzed fatty acids (FA), the ether extract was solubilized in KOH (1 mol/L) solution and kept under reflux for 30 minutes. After cooling, the solution was acidified with 1 mol/L hydrochloric acid (HCl) and extracted with hexane, which was removed in a rotary evaporator (Ika RV10). To obtain the fatty acids methyl esters (FAME), the fatty acids were solubilized in hexane, a 2% v/v methanolic solution of sulfuric acid (H_2SO_4) was added, which was kept under reflux for 1 hour. After cooling, the organic phase obtained with the addition of saturated sodium chloride solution (NaCl), and the hexane was removed in a rotary evaporator (Ika RV10) (Silva et al., 2015).

2.4 Phytochemical screening

Steroids and triterpenoids presence was evaluated by Lieberman-Burchard reaction, alkaloids by Dragendorff reaction and coumarins and anthraquinones by the addition of NaOH (1 mol/L) (Matos, 2009).

2.5 Evaluation of antioxidant activity

The antioxidant activity was evaluated by DPPH scavenging test (Brand-Williams, Cuvelier & Berset, 1995), with modifications (Araújo et al., 2013). Exactly 75 µL of the samples (1, 10, 100, 250, and 500 µg/mL) and 150 µL of the DPPH (0.0002% v/v) solution were added in a 96-well plate and maintained at 25 °C for 30 minutes. The reading was made on a spectrophotometer (Power Wave XS2, λ = 517 nm). The experiment was done in triplicate. The percentage of antioxidant activity was calculated using the formula: **% inhibition of DPPH = [1– (Aa / Ab)] x 100,** where **Aa** = absorbance of the sample and **Ab** = absorbance of DPPH.

The effective concentration to decolorize 50% of the DPPH solution (EC_{50}) was calculated using the probitos analysis method (Finney, 1980).

2.6 *Artemia salina* lethality assay

Saline water was prepared by adding 38 g of sea salt in 1 L of distilled water. *A. salina* eggs were placed in the solution (200 mg/400 mL) for 48 hours under aeration, being the first four hours under 40 w light. Then, 10 nauplii were collected and transferred to test tubes with the samples (1000, 500, 250 and 125 µg/mL) and DMSO (1, 0.5, 0.25 and 0.125%). The nauplii were counted with the aid of a magnifying glass after 24 hours of treatment with samples (Pimenta et al., 2003) and the Lethal Concentration capable of killing 50% (LC_{50}) and 90% (LC_{90}) was calculated by the probit analysis method (Finney, 1980).

3. Results and Discussion

3.1 Phytochemical screening

The presence of steroids, triterpenoids and alkaloids was observed in EE, which corroborates with the literature. The monoterpene alkaloids (tecomine and tecostanin), 5-*β*-hydroxyskitantine and boschniakina have already been identified in *T. stans* seeds and leaves (Costantino et al., 2003). Gonçalves, Parreira & Lima (2020) also related the presence of alkaloids and steroids in hexane fraction from flowers.

3.2 Evaluation of antioxidant activity

As shown in Table 1, the EE and FA exhibited antioxidant activity greater than 50% in all concentrations evaluated. The samples, at concentrations of 1 and 10 µg/mL, were more efficient in capturing the DPPH radical than the positive control BHT, with small values of EC_{50}.

Table 1. DPPH scavenging activity and EC_{50} values of ether extract and fractions from *Tecoma stans* seeds.

Samples	DPPH-scavenging activity					EC_{50} (µg/mL)[1]
	1 µg/mL	10 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL	
EE	51.96 ± 0.82*	52.75 ± 0.82*	55.92 ± 0.23*	58.03 ± 1.50*	65.69 ± 0.79*	0.92 ± 0.07*
FA	51.17 ± 0.60*	53.15 ± 0.60*	54.21 ± 0.68*	55.66 ± 0.60*	59.62 ± 4.92*	0.91 ± 0.08*
FAME	49.85 ± 0.39*	50.38 ± 2.32*	51.43 ± 2.20*	52.62 ± 1.81*	55.53 ± 0.99*	1.03 ± 0.10*
BHT	18.5 ± 0.14	25.58 ± 0.28	86.51 ± 0.61	91.37 ±0.16	94.19 ± 0.37	16.36 ± 1.63*

Ether extract (EE), fatty acids (FA), fatty acids methyl esters (FAME), 2,6-di-tert-butyl-4-methylphenol (BHT). [1]EC_{50}: Effective concentration (in µg/mL) of sample required to inhibit the formation of DPPH radicals by 50%. *: Statistically significant ($p < 0.05$) compared to BHT. The results were expressed in means ± SE (n = 3). Source: Authors.

No works were found for the antioxidant activity of *T. stans* seeds, so our results were compared with studies from other parts of the plant. The samples of flowers showed EC_{50} values of 3.76 and 2.99 µg/mL for the ethanol extract and the dichloromethane fraction, respectively (Gonçalves, 2020). The fruits extract obtained with water/ethanol (1:4), under reflux at 70 °C, presented inhibition concentration (IC_{50}) of 12.7 µg/mL (Marzouk et al., 2006). These results indicate that the increase in temperature does not influence the antioxidant activity (Simões et al., 2007).

Dry leaves extract, obtained by percolation with water/ethanol (1:1), and the fractions of petroleum ether, ethyl acetate and methanol showed antioxidant activity, being hydroethanolic extract the most active, with 64.32% of DPPH inhibition (Larbie, Nyarkoh & Adjei, 2019). Leaves and branches extracts obtained by maceration exhibited IC_{50} values between 10 and 100 µg/mL (Larbie, Nyarkoh & Adjei, 2019; Salem et al., 2013). The results obtained for the seeds were more promising than for the other plant parts.

The antioxidant activity of EE may be related to secondary metabolites observed. The cyclic carbons together with conjugated double bonds in steroids and terpenes make them potential reducing agents, because they can capture and stabilize Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) from the environment. Nitrogen atoms in alkaloids molecules have free electron pairs that can also stabilize ROS and RNS. The steroidal alkaloids presented high antioxidant potential (Cerqueira, de Medeiros & Augusto, 2007; Simões et al., 2007).

The antioxidant activity of FA and FAME of *T. stans* corroborates with the literature. FAME obtained from the seeds of *Annona cornifolia* (Annonaceae) presented IC_{50} = 3.83 µg/mL (Lima et al., 2012), as well as FAME from commercial soy, corn and sunflower oils exhibited IC_{50} values between 1 and 10 µg/mL (Pinto et al., 2017). A study with FA and FAME of leaves of *S. brasiliensis* showed IC_{50} values < 1 µg/mL (Amado et al., 2018).

3.3 *Artemia salina* lethality assay

As shown in Table 2, the EE promoted low mortality of *A. salina* in all concentrations, causing lethality below 20% in the highest concentration tested. FA caused mortality below 50% at concentration of 500 µg/mL; however, at concentration of 1000, the mortality was 91.11%. FAME exhibited mortality higher than 50% in all concentrations evaluated.

Table 2. *A. salina* letality, LC_{50} and LC_{90} values of ether extract and fractions from *Tecoma stans* seeds.

Samples	*Artemia salina* **letality**				**LC_{50}**	**LC_{90}**
	125 µg/mL	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**	**(µg/mL)[1]**	**(µg/mL)[2]**
EE	0.00 ± 0.00	2.22 ± 0.96[a]	4.44 ± 1.36[a]	8.88 ± 2.25[b]	N.D.	N.D.
FA	16.65 ± 2.96[a]	28.86 ± 3.67[b]	38.33 ± 4.19[b]	95.46 ± 2.27[c]	486.27	1180.94
FAME	67.71 ± 1.38[a]	88.80 ± 2.63[b]	98.86 ± 1.41[c]	100.00 ± 0.00[c]	7.52	164.63

Ether extract (EE), fatty acids (FA) and fatty acids methyl esters (FAME). [1]LC_{50}: Lethal concentration (µg/mL) of sample required to kill *A. salina* by 50%. [2]LC_{90}: Lethal concentration (µg/mL) of sample required to kill *A. salina* by 90%. The results were expressed in means ± SE (n = 3). Means followed by the same letter on the same line do not differ according to the Tukey test ($p < 0.05$). N.D.: not determined. Source: Authors.

A. salina is considered a good model for assessing the toxicity of plant extracts since the results obtained in this assay can be extrapolated to other tests (Pimenta et al., 2003). However, studies with FA and FAME assessing lethality in this model are still scarce.

FAME from *A. cornifolia* seeds promoted high mortality in *A. salina* nauplii, with LC_{50} = 8.77 µg/mL (Lima, 2006). Another study evaluating the lethality of EE, FA and FAME of *S. brasiliensis* leaves in *A. salina*, it was observed that FAME exhibited higher lethality (LC_{50} = 681.59 µg/mL), followed by FA (LC_{50} = 899.34 µg/mL), while FAME did not present considerable toxicity (Amado et al., 2018).

Studies evaluating the activity of *T. stans* on *A. salina* are even scarce, with only one report in the literature. Thein & Oo (2019) evaluated the larvicidal activity of aqueous and ethanolic extracts (70%) from leaves. For the aqueous extract, the concentrations between 187.5 and 3000 µg/mL were tested, with mortality ranging between 7% and 96%; for the ethanolic extract (70%), the concentrations were from 250 to 4000 µg/mL, with mortality between 3% and 91%. The LC_{50} values obtained were 878 µg/mL for the aqueous extract and 1318 µg/mL for the ethanolic extract (70%). In our study, the LC_{50} values found for FA and FAME of the seeds demonstrated greater toxicity than the aqueous and ethanol extracts (70%) of the leaves. Thus, as FA and FAME have LC_{50} values less than 1000 µg/mL, they are considered toxic; while EE has no toxicity (Meyer et al., 1982).

4. Final Considerations

In this study, it can be concluded that EE, FA and FAME from *T. stans* seeds have antioxidant activity, with potential for future studies. This effect may be related to the presence of steroids, triterpenoids and alkaloids. Among the samples, the EE is safe for the environment, while FA and FAME have toxicological potential.

In order to improve this study, it is interesting to identify the compounds present in EE, FA and FAME, and evaluate their effect in other biological models.

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