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Entomotoxicity of *Dysphania ambrosioides* (Amaranthaceae) and *Coronopus didymus* (Brassicaceae) hydroalcoholic leaf extracts assessed in cockroach semi-isolated heart preparation

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ABSTRACT

In this work, the cardiotoxicity of *Dysphania ambrosioides* and *Coronopus didymus* hydroalcoholic leaf extracts in cockroach semi-isolated heart preparation was assessed and their secondary metabolites were identified. The hydroalcoholic leaf extracts (0.2%) were obtained using a maceration method. The experiments were carried out using different concentrations (150, 300, 600 and 1200 µg) of these extracts in a final volume of 150 µL (n = 4) which were then added directly onto the preparations with heart rate (beats per min) being monitored under stereoscopy for 30 min. The extracts were phytochemically screened to identify their potential active compounds. In those preparations treated with *D. ambrosioides* and *C. didymus* extracts (150 and 300 µg) there was no alteration in heart rate whereas the highest concentrations (600 and 1200 µg) produced an immediate and potent blockade of the heart beats. The phytochemistry analysis revealed the presence of alkaloids and tannins as secondary metabolites in both extracts. In conclusion, *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts show potent cardiotoxicity in cockroach semi-isolated heart preparation, which may be associated with the alkaloids and/or tannins present in these extracts.

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KEYWORDS: *Dysphania ambrosioides*, *Coronopus didymus*, entomotoxicity, cockroach, heart rate, *ex vivo*.

1. INTRODUCTION

Brazil is known for its immense plant resource diversity. *Dysphania ambrosioides* (Amaranthaceae family) and *Coronopus didymus* (Brassicaceae family) are popularly known as 'Erva de Santa Maria' and 'Mentruz Rasteiro', respectively, and they are widely distributed in south and southeast regions of Brazil [1]. These plants are commonly used by the local population, especially from rural areas, as tea and as topical compresses to treat various skin injuries [2].

D. ambrosioides is characterized by ramified branches which can reach up to one meter height and are composed of single peciolated leaves that vary in size. *D. ambrosioides* releases a peculiar odour and its fruits are small containing an oily substance. The extract prepared from *D. ambrosioides* leaves has been used for treatment of a variety of diseases, including bronchitis and tuberculosis. In addition, *C. didymus* is a seasonal plant, most frequently occurring during the winter, and it exhibits ramified branches with pinnately compound leaves that are lobed along their margins, small flowers and has an odour much similar to watercress. *C. didymus* has flowers arranged in racemose inflorescence being able to reach more than thirty centimeters length. The leaf extract of *C. didymus* has been popularly

used to treat bronchitis, urinary tract and gastric infections, anaemia, diabetes and rheumatism [3].

Bioactive compounds isolated from a wide diversity of plants with potential therapeutic properties have been explored by pharmaceutical industries. However, high concentrations of plant extracts or their isolated compounds are considerably toxic being able to induce serious systemic complications. Although plant extracts and their isolated compounds have shown beneficial effects through their use in the treatment of a range of diseases and injuries in general, their inappropriate use can produce systemic toxicity. In addition, the toxic mechanisms of these therapeutic tools have been poorly investigated in the last decades. Based on the indiscriminate use of these plant extracts and their isolated compounds, systematic investigations are still necessary in order to exactly understand the relationship between the ideal therapeutic concentration of these extracts and their potential toxicity [4].

In this work, the cardiotoxicity of *Dysphania ambrosioides* and *Coronopus didymus* hydroalcoholic leaf extracts in cockroach semi-isolated heart preparation was assessed and their secondary metabolites were identified. The lack of data about the entomotoxicity of these plants supports the purpose of this investigation which may contribute to understand their pharmacological applicability [5].

2. MATERIALS AND METHODS

2.1. Botanical material

Dysphania ambrosioides and *Coronopus didymus* were obtained from Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP, Sorocaba, SP, Brazil). The plants were cleaned and weighted (‘*D. ambrosioides*’- 7.1 g and ‘*C. didymus*’ - 6.7 g) before drying for 16 hours at 60 °C in a drying oven incubator; the dried leaves were subsequently triturated to obtain a powdered material of 0.75 g and 1.24 g, respectively.

2.2. *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts

The powdered materials obtained from dried leaves of *D. ambrosioides* and *C. didymus* were solubilized in ethanol 70% (1 g of powdered material incubated with 20 mL of hydroalcoholic solution for 48 hours resulted in a hydroalcoholic extract (0.2%)), following a conventional method essentially described elsewhere [6].

2.3. Cockroach semi-isolated heart preparation

The cockroaches (*Nauphoeta cinerea*) were purchased from Pet Shop Bicho & Cia (Sorocaba, SP, Brazil) and maintained in an appropriate container at room temperature (23–25 °C) in a 12:12 h light/dark cycle until use; the animals had free access to water and food (pieces of fruits and vegetables) *ad libitum*. The cockroaches were lightly anesthetized with CO_2 (saturated atmosphere) and then placed dorsal surface down under a dissecting microscope, followed by immobilization by entomological pins. The lateral margins of the abdomen were cut along each side and the ventral cuticle removed to expose the viscera. The viscera were then carefully moved to one side in order to expose the vascular system attached to the dorsal body wall [7]. The heart contractions were observed under a magnification of 30x using an appropriate stereoscopy (Carl Zeiss, Germany). In some preparations, the heart rate was monitored for 60 min only in the presence of saline solution at T_0 (basal), T_5, T_{15}, T_{30} and T_{60} min. In those preparations exposed to extracts, the basal (T_0) heart rate was monitored before adding the extract of *D. ambrosioides* or *C. didymus* (150, 300, 600 and 1200 µg in a final volume of 150 µL each) and then in the intervals T_5, T_{15}, T_{30} and T_{60} min [8].

2.4. Phytochemistry screening of the compounds present in the plant extracts

The determination of secondary metabolites presents in the *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts was performed as essentially recommended by Sociedade Brasileira de Farmacognosia (Curitiba, PR, Brazil) to identify the following compounds: tannin, flavonoid, alkaloid, anthraquinone and saponin.

2.5. Statistical analysis

Changes in the heart rate of cockroach semi-isolated heart preparations were expressed as a percentage relative to basal values. The results were expressed as the mean ± SEM and statistical comparisons were done using Student’s *t*-test or analysis of variance (ANOVA), with $p < 0.05$ indicating significance.

3. RESULTS

In preparations exposed to *D. ambrosioides* hydroalcoholic leaf extract (150 and 300 µg), there was no significant alteration in heart rate compared to basal values or control preparations; however, the highest extract concentrations (600 and 1200 µg) abolished the heart contractions at 5-min exposure time (Figure 1). *C. didymus* hydroalcoholic leaf extract also did not affect the heart contractions at the lowest concentrations (150 and 300 µg) tested in cockroach semi-isolated heart preparation; the highest extract concentrations (600 and 1200 µg) caused complete blockade of the heart contractions at 5-min exposure time, as similarly observed with *D. ambrosioides* extract (Figure 2). Table 1 summarizes the presence of phytochemical compounds found in the *D. ambrosioides* and *C. didymus* extracts; note that both extracts contain tannins and alkaloids, while *D. ambrosioides* extract only contains anthraquinones.

4. DISCUSSION

In a recent study, the positive and negative chronotropic activity of adrenaline (sympathomimetic action) and acetylcholine (parasympathomimetic action), respectively, on cockroach semi-isolated heart preparation has already been demonstrated in order to understand the mechanisms by which *Nephrolepsis exaltata* extract induces toxicity on these preparations; the cardiotoxicity induced by *Nephrolepsis exaltata* extract has been associated with terpenes, a predominant phytochemical compound in this extract [9]. Here, it has been observed that the *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts did not show concentration-dependence in causing blockade of the heart contractions, in contrast to the findings with *Nephrolepis exaltata* aqueous extract which showed concentration-dependent blockade of the contractions in cockroach semi-isolated heart preparation [9]. In cockroach semi-isolated heart preparation, the entomotoxic concentrations for both of extracts were 600 and 1200 µg with a complete blockage of the heart contractions occurring at five-min exposure time.

The phytochemical analysis revealed the presence of alkaloids in both the extracts; alkaloids isolated from seeds of *Erythrina americana* (Fabaceae) induced high mortality in larvae of *Culex*

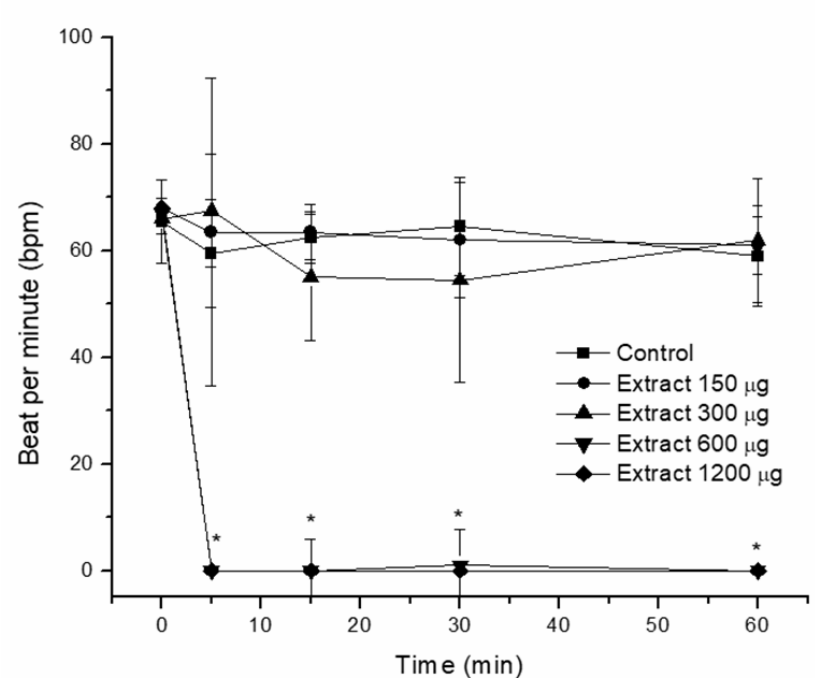

Figure 1. Effect of *D. ambrosioides* hydroalcoholic leaf extract on the heart rate monitored in cockroach semi-isolated heart preparation. The points are the mean ± SEM (n = 4); *$p < 0.05$ compared to control preparations.

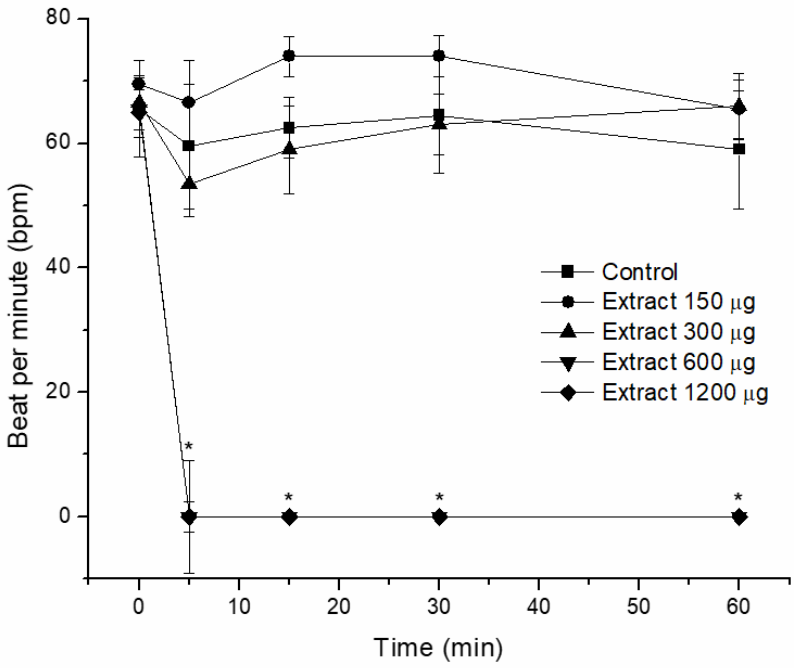

Figure 2. Effect of *C. didymus* hydroalcoholic leaf extract on the heart rate monitored in cockroach semi-isolated heart preparation. The points are the mean ± SEM (n = 4); *$p < 0.05$ compared to control preparations.

Table 1. Phytochemical analysis of the *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts.

Active principle	*C. ambrosioides*	*C. didymus*
Tanines	Positive	Positive
Flavonoids		
- Shinoda Reaction	Negative	Positive
- Ferric chloride	Positive	Negative
- Sodium hidroxide	Negative	Negative
- Aluminum chloride	Positive	Negative
Alkaloids		
- Dragendorff	Positive	Positive
- Bertrand	Positive	Positive
- Mayer	Positive	Positive
- Bouchardat	Negative	Negative
Antraquinons	Positive	Negative
Saponins	Negative	Negative

quinquesfasciatus [10]. In addition, an alkaloid isolated from *Unonopsis lindmanii* (Annonaceae) hydroalcoholic stem extract caused toxicity on the microcrustacean *Artemia salina* [11]. The cytotoxicity induced by alkaloids isolated from *Annona montana* (Annonaceae) leaf extract on lung, cervix and KB-type tumor cells has already been reported [12]. On the other hand, plant extracts characterized by the presence of tannins showed potent molluscicidal activity [13, 14].

Recently, an investigation on *D. ambrosioides* leaf extract determined some types of secondary metabolites: 1) high concentration of ascaridole exhibiting vermifuge-like activity, 2) presence of flavonoids with potential antioxidant activity and 3) tannins with potent anti-inflammatory action [15]. It has also been reported that high concentrations of *D. ambrosioides* leaf extract can cause peristaltic dysfunctions, respiratory failure and hypotension in humans [15]. Therefore, due to the risks associated with the cardiovascular alterations induced by *D. ambrosioides* leaf extracts in humans, as previously reported, it is necessity to investigate the pharmacology of the isolated compounds directly involved in the extract-induced blockade in cockroach semi-isolated heart preparation before proposing to use it for developing new biopesticides [16]. The secondary metabolites from *C. didymus* are similar to those present in *D. ambrosioides* with both of them being composed by flavonoids, glycosides and essential oils that are predominantly present in their leaves and seeds. *C. didymus* aqueous extract also proved to be efficient in improving the process of wound healing in rats, promoting significant increase of fibroblasts and collagen fibers [17].

D. ambrosioides and *C. didymus* hydroalcoholic leaf extracts demonstrated a potential insecticidal action modulated most likely by alkaloids and tannins. However, the structural and pharmacological characterisation of new molecules from plant extracts with potential insecticidal activity has not been fully explored. Therefore, new investigations in order to improve our understanding of the mechanism of action of such molecules represent an essential pathway to develop new biopesticides [18].

5. CONCLUSION

In conclusion, *D. ambrosioides* and *C. didymus* hydroalcoholic leaf extracts induced potent entomotoxicity in cockroach semi-isolated heart preparation; both extracts were more active at the highest concentrations tested in this preparation. The cardiotoxicity produced by *D. ambrosioides* and *C. didymus* may be attributed to the presence of alkaloids and tannins in these extracts.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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