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Phytotoxic activity of compounds from *Moutabea guianensis* aubl. on Amazonian invasive species

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Resumo: Este estudo teve como objetivo estabelecer as variações na atividade fitotóxica dos extratos hexânico, acetato de etila e metanólico das raízes de *Moutabea guianensis*, e das substâncias cafeato de metila e escopoletina isoladas do extrato acetato de etila, variando a concentração e as espécies receptores. Foram desenvolvidos bioensaios de atividades fitotóxicas de germinação (a 25 °C e 12 horas de fotoperíodo) e de desenvolvimento da radícula e do hipocótilo (25 °C e 24 horas de fotoperíodo). A germinação das sementes de *Mimosa pudica* foi sensível aos extratos hexânico, acetato de etila e metanólico a 1% (w/v), com efeitos de inibição em 92%, 100% e 100%, respectivamente. A análise comparativa da atividade fitotóxica das substâncias testadas revelou que a escopoletina apresentou um potencial de inibição mais elevado no bioensaio de germinação de sementes frente a *Mimosa pudica*. *Senna obtusifolia* não foi sensível às substâncias testadas. Cafeato de metila apresentou maior potencial de inibição no bioensaio de desenvolvimento da radícula e hipocótilo, e a intensidade dos efeitos alelopáticos variou com as concentrações.

Palavras-chave: fitotóxico, *Moutabea guianensis*, escopoletina, metilcafeato, ervas

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Abstract: This study aimed establish the variations in the phytotoxic activity of hexane, ethyl acetate and methanol extracts of *Moutabea guianensis*, and methyl caffeate and scopoletin isolated from the ethyl acetate extract, variyng the concentration and the receptor species. Phytotoxic activity bioassays of germination (at 25 °C and 12 hours of photoperiod) and development of radicle and hypocotyl (25 °C and 24 hours of photoperiod) were developed. The seed germination of *Mimosa pudica* was sensitive to the roots hexane, ethyl acetate and methanol extracts at 1% (w/v), with inhibition potentials in 92%, 100% and 100%, respectively. Comparative analysis on the phytotoxic activity of the tested compounds revealed that scopoletin showed a higher inhibition potential on the seed germination bioassay against *Mimosa pudica*. *Senna obtusifolia* was not sensitive to the tested compounds. Methyl caffeate showed the highest potential to inhibit the development of radicle and hypocotyls, and the intensity of the allelopathic effects varied with the concentrations.

Keywords: phytotoxic, *Moutabea guianensis*, scopoletin, methyl caffeate, weeds

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INTRODUCTION

An alternative for replacing the current forms of weed control has been using plants and microorganisms for obtaining phytotoxic compounds, to be used directly as herbicides, or as prototypes for discovering new synthetic herbicides [1,2]. Natural products have been long used on the development of herbicides with new action mechanisms, and the natural phytotoxins frequently present sites of action different from those targets of common synthetic herbicides [3]. The natural phytotoxins present wide structural diversity with compounds belonging to various classes of substances [4]. The Amazon, due to its diversity of the flora constitutes a large reservoir of substances which may contribute to the number of compounds with phytotoxic activity and can lead to future products that may replace or reduce the use of existing defensive pesticides.

Moutabea guianensis is an Amazonian species that belongs to the family Polygalaceae, commonly known as cipó gogó de guariba [5]. Xanthones [6], coumarins [7], triterpenoids [8,9] steroids [10], flavonoids [11], pyrones [12,13] and xanthones [14,15] have been isolated from the species of the family Polygalaceae. In this study, from the roots of M. guianensis were isolated two compounds, the phenylpropanoid derivative methyl caffeate (1) and the coumarin scopoletin (2). Coumarins represent a kind of substances with inhibitory activity reported on the seed germination [16], and the phenylpropanoids and its derivatives have also been reported in the literature for presenting phytotoxic activity [17,18]. Thus, as part of a program on Amazonian plants with allelopathic and phytotoxic activity [19,20], in this work we report the phytotoxic activity of extracts and compounds from the roots of Moutabea guianensis against two invasive plants Mimosa pudica (malicia) and Senna obtusifolia (mata-pasto).

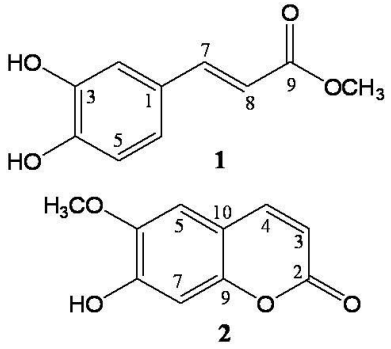

Figure 1 - Structure of the Methyl Caffeate (**1**) and scopoletin (**2**).

EXPERIMENTAL PROCEDURES

Plant material

Roots of *Moutabea guianensis* were collected in the experimental field of Embrapa Amazônia Oriental, located in Belém, Pará State, in March 2009. One voucher specimen is cataloged in the herbarium of Museu Paraense Emílio Goeldi (Belém, Brazil), under the number 195862.

Procedures for obtaining crude extracts and isolation of compounds 1 and 2

The air-dried ground roots of *M. guinensis* (2.5 kg) were submitted to several extractions with hexane, ethyl acetate and methanol solvents. Hexane (5.0 g), ethyl acetate (28.0 g) and methanol (48.3 g) extracts were obtained, respectively. The ethyl acetate extract was fractionated by column chromatography (CC) using silica gel as adsorbent eluted with the gradient systems: hexane-ethyl acetate from 90:10 to 0:100 (v/v), affording 24 fractions (each 200 mL). The eluted fractions were evaluated and grouped according to TLC analysis, to afford 8 groups of fractions. Group 3 (646 mg) was purified by recrystallization with methanol- chloroform (50:50) to yield 1 (523 mg). Group 4 (824 mg) was also purified by recrystallization from methanol-chloroform (50:50) to give 2 (690 mg). The structural elucidation of isolated compounds was performed by analysis of ^{1}H and ^{13}C NMR spectra obtained in methanol (CD_3OD) for 1 and chloroform ($CDCl_3$) for 2 and comparison with literature data.

Bioassay for phytotoxic evaluation

The evaluations of the effects of potentially phytotoxic extracts and substances methyl caffeate (1) and scopoletin (2) were performed to seed germination and radicle and hypocotyl growth of weed plant *Mimosa pudica* and *Senna obtusifolia*.

Seed germination bioassay with extracts

The weed seeds were collected in areas cultivated pastures at the municipality of Castanhal-PA. Later, they passed through a cleaning process and were treated with concentrated sulfuric acid, in order to break dormancy, as established by Souza Filho [21].

The bioassay for evaluation of phytotoxic activity of hexane, ethyl acetate and methanol extract on seed germination were monitored for a 7 days period, with daily counts and elimination of seeds germinated. Germinated seeds were considered those who presented root length less than 2.0 mm [22]. The bioassay was developed in conditions of 25 °C constant temperature and a photoperiod of 12 h. Each Petri dish of 9 cm diameter, lined with filter paper quality, received 3.0 mL of the solution prepared with extracts. Each solution was prepared with the same solvent that the extracts were obtained. After solvent evaporation, each plate received 20 seeds and subsequently distilled water in the same amount of solutions, keeping the original concentration. The extracts were tested at a concentration of 1% (w/v). The germination bioassay was performed in triplicate.

Seed germination bioassay with substances

The seed germination bioassay performed with the substances methyl caffeate and scopoletin were performed under the same conditions for bioassay seed germination with extracts. However, the substances were tested at concentrations of 10 and 55 mg/L.

Statistical analysis

The experimental design for all bioassays was completely randomized with three replications. Distilled water was used as control treatment. The data were subjected to analysis of variance (F test) and the means compared by the Tukey test (5%). All the analysis was carried out by the SAS software [23].

RESULTS AND DISCUSSION

Hexane, methanol and ethyl acetate extracts from the roots of *M. guianensis* showed high potential inhibition in phytotoxic bioassays. The phytotoxic bioassays were also conducted with 1 and 2, isolated from the ethyl acetate extract. From the analysis of the ^{1}H and ^{13}C NMR spectra and subsequent comparison with data obtained from the literature (Table 1 and 2), compounds 1 and 2 were identified, respectively, as methyl caffeate [24] and scopoletin [25] (Figure 1).

Table 1: ^{13}C NMR data (75 MHz, $CDCl_3$) of **1** in comparison with ^{13}C NMR literature data (125 MHz, $CDCl_3$)[22].

Position	δ_C**1**	δ_C (Lit.)[22]
1	127.5	126.7
2	115.1	115.3
3	146.2	148.2
4	148.7	149.3
5	116.3	114.7
6	122.5	122.8
7	145.7	145.7
8	115.3	114.7
9	167.8	169.8
OCH_3	51.7	55.3

Table 2: ^{13}C NMR data (75 MHz, CD_3OD) of **2** in comparison with ^{13}C NMR literature data (50 MHz, CD_3COCD_3)[23].

Position	δ_C**2**	δ_C (Lit.)[23]
2	161,2	161.8
3	113,3	113.1
4	144,6	143.0
5	109,9	107.9
6	145,9	144.3
7	151,7	150.8
8	103,6	102.9
9	151,1	148.6
10	112,0	110.3
OCH_3	56,7	56.8

Phytotoxic effects of the extracts

The seed germination of *Mimosa pudica* was sensitive to the roots extracts of *M. guianensis* at 1% (w/v). The inhibitory effects were 92%, 100% and 100%, respectively, for the hexane, ethyl acetate and methanol extracts. When *Senna obtusifolia* was used as receptor species, the highest inhibition were 59% and 51%, respectively, for the hexane and methanol extracts. The results of the seed germination bioassays with the extracts are shown in figure 2.

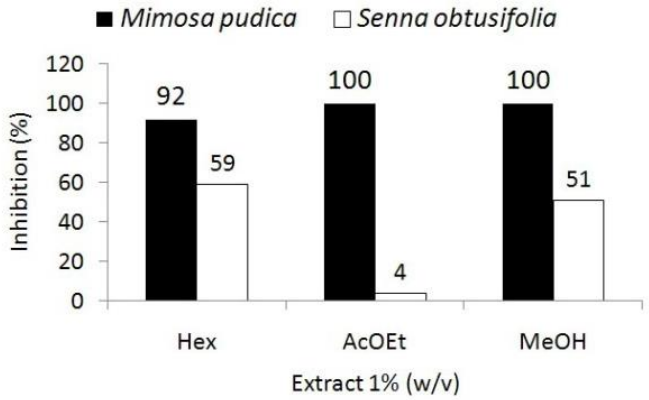

Figure 2 - Effects of hexane, ethyl acetateand methanol extracts (1% w/v) from the roots of *Moutabea guianensis* on seed germination of *Mimosa pudica* and *Senna obtusifolia*. Data expressed as percentage inhibition compared to control treatment.

Phytotoxic effects of substances

At 55 mg/L, the substance scopoletin (2) showed a greater potential inhibition (49%) on seed germination bioassay against *M. pudica*. Methyl caffeate (1) also showed maximum inhibitory effect at the highest concentration tested (55 mg/L), which was 18% against *M. pudica* (Figure 3). The inhibitory effects on the seed germination of *M. pudica* was dependent on the concentration of the substances, since the phytotoxic effects increased significantly with increasing concentration from 10 to 55 mg/L.

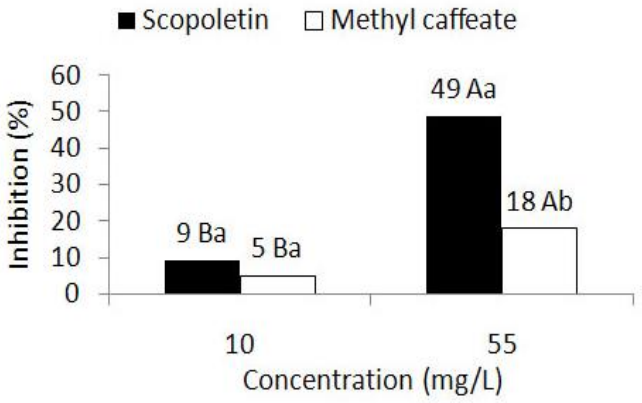

Figure 3 – Effects of compounds on the seed germination of *Mimosa pudica* (A). Data expressed as percentage inhibition compared to control treatment. Means followed by the same letters, upper case combinations of 1 and 2 and sensitive to the inhibitory effect between species are not statistically different by Tukey test ($p>0.05$).

Scopoletin (2) showed a higher inhibition effect on the seed germination of *M. pudica* than methyl caffeate, and this fact is an indication that this substance may be responsible for the inhibition of seed germination of the ethyl acetate extract. Scopoletin is a substance that belongs to the class of coumarins, which are described in the literature as potent inhibitors of seed germination [16], besides that scopoletin specifically has been previously described in the literature as a potent allelochemical [26-28]. Jacobi and Fleck [29] showed that oat genotypes with high phytotoxic activity against ryegrass and wheat were those that exude higher amounts of scopoletin.

Both substances showed stimulatory activity (Figure 4) when tested against *S. obtusifolia*.

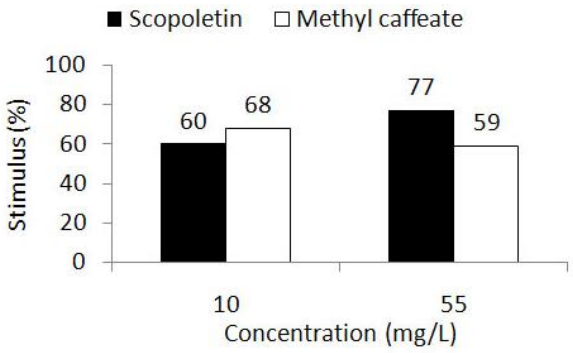

Figure 4 – Effects of compounds on the seed germination of *Senna obtusifolia* (B). Data expressed as percentage inhibition compared to control treatment. Means do not differ per Tukey test ($p>0.05$).

CONCLUSIONS

The studies showed that scopoletin have potent phytotoxic activity. Scopoletin is the most active substance in the seed germination assay with *M. pudica*. On the other hand, seed germination of *S. obtusifolia* was not sensitive to the substances. The isolament of scopoletin and methyl caffeate from *M. guianensis* opens new possibilities for studies aiming to enhance the phytotoxic effects of these substances or the use of these substances in the control of the invasive species *M. pudica* and *S. obtusifolia*, which has caused much damage to the Amazon's agricultural sector.

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