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Original Article

Phytotoxicity of organic extracts of five medicinal plants of the Neotropical savanna

Fitotoxicidade de extratos orgânicos de cinco plantas medicinais do Cerrado

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Abstract

Medicinal plants produce a high diversity of secondary metabolites with different biological activities, which are commonly evaluated when prospecting for bioherbicides. We analyzed the phytotoxic activity of organic extracts from the leaves of five medicinal species, *Byrsonima intermedia*, *Moquiniastrum polymorphum*, *Luehea candicans*, *Miconia chamissois*, and *Qualea cordata*. Phytotoxicity was evaluated on the initial growth of cucumber seedlings through tests with different concentrations of hexane, ethyl acetate, and methanol extracts. The results showed that all organic extracts and all concentrations affected cucumber development, with methanol extracts generally showing the greatest negative effect on the initial growth of the target species. The only exception was for *M. chamissois* extracts, in which the hexane extract had the greatest phytotoxicity. Furthermore, the organic extracts were subjected to preliminary phytochemical analysis, revealing the widespread presence of alkaloids along with other chemical classes. All the study species are thus potential candidates for use as natural herbicides.

Keywords: Cerrado, cucumber initial growth, leaf extract, phytochemical screening.

Resumo

As plantas medicinais são comumente avaliadas na prospecção de bioherbicidas, por produzirem uma alta diversidade de metabólitos secundários com diferentes atividades biológicas. Analisamos a atividade fitotóxica de extratos orgânicos de folhas de cinco espécies medicinais, *Byrsonima intermedia*, *Moquiniastrum polymorphum*, *Luehea candicans*, *Miconia chamissois* e *Qualea cordata*. A fitotoxicidade foi avaliada no crescimento inicial de plântulas de pepino por meio de testes com diferentes concentrações de extratos de hexano, acetato de etila e metanol. Os resultados mostraram que todos os extratos orgânicos e todas as concentrações afetaram o desenvolvimento de pepino, com os extratos metanólicos geralmente apresentando a maior inibição no crescimento inicial da espécie-alvo. A única exceção foram os extratos de *M. chamissois*, em que o extrato hexânico apresentou a maior fitotoxicidade. Adicionalmente, os extratos orgânicos foram submetidos a análises fitoquímicas preliminares, revelando a presença generalizada de alcaloides, juntamente com outras classes químicas. Todas as espécies estudadas são, portanto, potenciais candidatas para uso como herbicidas naturais.

Palavras-chave: Cerrado, crescimento inicial de pepino, extrato foliar, triagem fitoquímica.

1. Introduction

The Cerrado is the second-largest Brazilian biome, the largest savanna in South America, and home to many medicinal plants. Among these, *Byrsonima intermedia* A. Juss (Malpighiaceae), locally known as "*murici*," has antiseptic, anti-hemorrhagic, antimicrobial, anti-diarrheal, anti-inflammatory, healing, and antimutagenic properties (Michelin et al., 2008; Guilhon-Simplicio and Pereira, 2011; Moreira et al., 2011), while *Moquiniastrum polymorphum* (Less.) G. Sancho (Asteraceae), popularly known as "*cambará*," has antioxidant, antimicrobial, anti-inflammatory, antiviral, and anticancer properties (Gonçalves et al., 2019). The chemical composition and the pharmacological and toxicological activities of *M. polymorphum* have been studied for the development of new medicinal drugs and insecticides (Rojas de Arias et al., 1995; Simões et al., 1999; Moreira et al., 2000; Gonçalves et al., 2019). Another medicinal species from the Cerrado is *Luehea candicans* Mart. (Tiliaceae), popularly known as "*açoita-cavalo*," which has potent antiproliferative activity in human kidney cancer cells (Silva et al., 2012). *Miconia chamissois* Naud. (Melastomataceae), commonly known as "*folha-de-bolo*," is considered a natural source of

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bioactive molecules for the development of new anticancer drugs (Silva et al., 2020; Rosa et al., 2021), while *Qualea cordata* Spreng. (Vochysiaceae), known as "*carvãozinho*", has antioxidant properties (Carnevale Neto et al., 2013) as well as antimicrobial, antiulcer, anticonvulsant, and analgesic activity (Carli et al., 2013).

Thanks to their production of a high variety of secondary metabolites (Qasem, 2002), medicinal plants are frequently also used as natural herbicides (Teerarak et al., 2010; Petrova et al., 2015; Algandaby and El-Darier, 2018). Weeds have a major impact on agriculture and are a leading cause of drops in food quality and agricultural productivity (Reddy et al., 2019). As cases of herbicide resistance increase worldwide, there is an increasing demand for new substances that are effective and inexpensive with a low environmental impact. Natural phytotoxins often act on unexplored molecular targets (Duke et al., 2000; Reigosa et al., 2013) or unlike synthetic ones (Dayan et al., 2009). The phytotoxicity of aqueous extracts from the five Cerrado species mentioned earlier – *B. intermedia*, *M. polymorphum*, *L. candicans*, *M. chamissois*, and *Q. cordata* – have been established in the literature (Pinto and Kolb, 2016). However, as the solvent polarity partially controls the solubility of chemical compounds, meaning that solvents directly influence the effectiveness of metabolite extraction, with organic solvents often more effective than aqueous ones (Cecchin et al., 2017), studies conducted with these solvents become important for a better understanding of the phytotoxicity of the species.

Therefore, this study aims to evaluate the phytotoxic effects of different organic extracts to characterize their composition and level of phytotoxicity and analyze the effects of different concentrations and solvents with varying levels of polarity on the early growth of cucumber seedlings.

2. Materials and Methods

2.1. Botanical material and extraction method

Healthy and completely expanded leaves of *Byrsonima intermedia*, *Moquiniastrum polymorphum*, *Luehea candicans*, *Miconia chamissois*, and *Qualea cordata* were collected from at least five randomly chosen individuals in the rainy season in the Ecological Station of Assis, municipality of Assis, São Paulo, Brazil (22°33'20" to 22°37'41"S and 50°24'48" to 50°21'27"W). The species were identified by Dr. Renata Giassi Udulutsch. Voucher specimens of *B. intermedia* (HASSI 2586), *M. polymorphum* (HASSI 2587), *L. candicans* (HASSI 2588), *M. chamissois* (HASSI 2589), and *Q. cordata* (HASSI 2590) were deposited at Herbarium Assisense (HASSI), Laboratory of Plant Systematics, Biological Sciences, Universidade Estadual Paulista, UNESP, Assis, SP, Brazil, and registered in SisGen within registration number AFD1429. Afterward, leaves were dried in the shade, ground in a Wiley mill, and submitted to maceration in the dark, with mechanical agitation in hexane for two hours at 24 °C in a ratio of 1:10 w/v (dried leaves: hexane). The procedure was repeated two more times with the same plant material. After complete maceration, the extract was vacuum filtered through 14 µm porosity qualitative filter paper (Qualy), and the solvent evaporated to obtain the hexane extract. The plant residue was then extracted three times with ethyl acetate using the same procedure. The plant residue resulting from these extractions was extracted three times with methanol. The organic extracts were solubilized in their respective solvents to obtain concentrations of 1.25, 2.5, 3.17, and 5.00 mg mL^{-1} for the phytotoxicity bioassays.

2.2. Physicochemical attributes of extracts

To eliminate possible interference during the bioassays, the physicochemical attributes of the crude organic extracts were determined: pH (with a pH meter, Tecnal, model TEC-2) and osmotic potential (using an Abbe refractometer with PEG 6000 solutions with different osmotic potentials). They were calculated with the following equation: refractive index in Brix = $-3.7765x^2 - 10.237x + 0.195$ (Villela et al., 1991).

2.3. Bioassays with organic extracts in Cucumis sativus L.

A total of 10 mL of each organic extract in hexane, ethyl acetate, and methanol at concentrations of 1.25, 2.5, 3.17, and 5.00 mg mL^{-1} were added to Petri dishes (100 mm diameter) containing two sheets of filter paper. The solvent was evaporated for 24 h at room temperature and protected from light, and then 10 mL of distilled water and 20 previously germinated cucumber seeds (2 mm of root protrusion) were added (three dishes, n = 60). Cucumber was used as a model seedling since it is highly sensitive to phytotoxicity bioassays (Rice, 1979; Ferreira and Aquila, 2000). Distilled water was used as a negative control. The Petri dishes were incubated in a germination chamber (model 102 FC; Eletrolab) at 25 ± 1 °C with a photoperiod of 12 h for 120 h. The length of the seedlings' main root and shoot were then measured. The growth parameters were calculated as the mean percentage difference compared to the negative controls (Chung et al., 2001) using the following Formula 1:

$$\text{Percentage difference}(\%) = \frac{\text{negative control} - \text{organic extract}}{\text{negative control}} \text{x}100 \quad (1)$$

Positive values represent stimulation, while negative values represent inhibition relative to the control.

2.4. Preliminary phytochemical analysis

A qualitative phytochemical analysis was performed on the hexane, ethyl acetate, and crude methanol extracts to determine the main chemical classes of the secondary metabolites (alkaloids, anthraquinones, coumarins, steroids, triterpenoids, flavonoids, saponins, and tannins) following Matos (1997).

2.5. Statistical analysis

The experimental design was completely randomized. The normality of the residuals (Shapiro-Wilk test, $p>0.05$) and the homogeneity of variances (Cochran test, $p>0.05$) of the data were tested. A one-way ANOVA followed by Dunnett's test ($p<0.05$) was used to determine significant differences between the organic and control extracts, and a factorial ANOVA followed by Tukey's test ($p\leq0.05$) was

performed to assess the interaction between the effects of organic extracts and concentrations. All statistical analyses were performed on absolute values.

3. Results

3.1. Physicochemical attributes of extracts

The pH values of the organic leaf extracts (hexane, ethyl acetate, and methanol) ranged from 3.90 to 6.35, while the potential osmotic values of the extracts ranged from -0.0005 to 0.0019 MPa. These values are within the ideal limits described in the literature (Eberlein, 1987; Pinto and Kolb, 2016), meaning that these factors did not affect the extracts' phytotoxicity.

3.2. Organic extract bioassays on cucumber seedlings

The statistical analyses showed significant differences in cucumber growth parameters across organic leaf extracts and concentration levels, as well as with respect to the interaction between the two. However, there was no significant interaction between extract and concentration for cucumber shoot length with *Q. cordata* extracts. For this species, there were differences across organic extracts, with the methanol extract being the only one to cause inhibition, and across concentration levels, with the lowest concentration of extract causing the greatest inhibition (data not shown).

All organic extracts and concentrations of *B. intermedia* significantly inhibited main cucumber root length except for hexane extracts at 1.25 and 2.50 mg mL^{-1}, which stimulated root growth, and ethyl acetate extract at 1.25 mg mL^{-1}, which did not affect root growth compared to the control (Table 1). The greatest inhibition of cucumber main root length was observed in the presence of methanol extracts at a concentration of 2.50 mg mL^{-1}. The organic extracts had no significant effect on cucumber shoot length, except for the ethyl acetate extract at 2.50 mg mL^{-1}, which led to a 13% increase compared to the control (Table 1).

All organic extracts of *M. polymorphum* significantly inhibited cucumber main root length at all concentrations, except for hexane extracts at 1.25 and 2.50 mg mL^{-1}, which had no effect relative to the control (Table 1). The greatest inhibition of main root length was observed in the presence of the methanol extract at a concentration of 5.00 mg mL^{-1}. The organic extracts had no significant effect on cucumber shoot length, except for the ethyl acetate extract at 5.00 mg mL^{-1} (Table 1).

The methanol and ethyl acetate extracts of *L. candicans* significantly inhibited the cucumber main root length at all concentrations compared to the control (Table 1). The greatest inhibition of main root length was observed in the presence of methanolic extracts at concentrations of 3.75 and 5.00 mg mL^{-1}. The hexane extracts did not affect root development compared to the control (Table 1). Organic extracts had no significant effect on cucumber shoot length, except for the hexane extract at 5.00 mg mL^{-1}, which stimulated shoot growth, and the ethyl acetate extract at 1.25 mg mL^{-1}, which inhibited shoot growth compared to the control (Table 1).

The hexane and methanol extracts of *M. chamissois* significantly inhibited cucumber main root length at all concentrations, except for hexane extract at 1.25 mg mL^{-1}, which did not affect root growth compared to the control (Table 1). The ethyl acetate extracts had no significant effect on the length of the main root of the target species, except at a concentration of 5.00 mg mL^{-1} (Table 1). The greatest inhibition of cucumber main root length was observed in the presence of the hexane extract at 5.00 mg mL^{-1}. The organic extracts had no significant effect on cucumber shoot length, except for the hexane extracts at 3.75 and 5.00 mg mL^{-1} and the methanol extract at 5.00 mg mL^{-1}, which inhibited shoot growth in comparison to the control (Table 1).

The ethyl acetate and methanol extracts of *Q. cordata* significantly inhibited cucumber main root length at all concentrations, except for the ethyl acetate extract at 1.25 mg mL^{-1}, which was not different from the control. The hexane extracts had no significant effect on the main root length, except for a concentration of 1.25 mg mL^{-1} (Table 1). The greatest inhibition of main root length was observed in the presence of the methanol extract at 5.00 mg mL^{-1}. Organic extracts had no significant effect on shoot length, except for the hexane extract at 5.00 mg mL^{-1}, which boosted shoot growth, and the methanolic extract at 1.25 mg mL^{-1}, which reduced shoot length compared to the control (Table 1).

3.3. Preliminary phytochemical analysis

Phytochemical screening was performed on the organic extracts of the five Cerrado species studied for eight classes of secondary metabolites (Table 2), of which only alkaloids were detected in the hexane extract of *M. chamissois*. Meanwhile, the methanolic extracts of at least one Cerrado species were positive for alkaloids, anthraquinones, coumarins, steroids, triterpenoids, flavonoids, saponins, and tannins.

Only the methanol extract of *L. candicans* tested positive for coumarins, while flavonoids were detected in the methanol extracts of *M. polymorphum* and *L. candicans* (Table 2). Meanwhile, triterpenoids, saponins, and tannins were found in the methanol extracts of *B. intermedia*, *L. candicans*, and *Q. cordata* (Table 2). The methanol extracts of *B. intermedia*, *M. chamissois* and *Q. cordata* showed positive results for alkaloids and anthraquinones. In addition, the presence of steroids was confirmed in the methanolic extract of *M. polymorphum* (Table 2).

4. Discussion

We extracted secondary metabolites in a series of solvents with increasing polarity to determine the effects of organic extracts of five representative medicinal species from the Cerrado. The data show that the methanolic extracts of *B. intermedia*, *M. polymorphum*, *L. candicans*, *Q. cordata*, and the hexane extract of *M. chamissois* were the most phytotoxic on the initial growth of cucumber seedlings. The effects of organic extracts on root development were more pronounced than on shoot growth. Root growth is generally suggested as a better indicator of

Table 1. Effect of concentration of organic extracts from leaves of medicinal species of Cerrado on the growth of cucumber seedlings. Data of extracts are presented as the mean percentage difference compared with the negative control (=100%) ± standard deviation. Absolute values for the negative control (distilled water) were presented in cm units' ± standard deviation.

Species	Extract	Concentration (mg mL^{-1})	Main root length (%)	Shoot length (%)
Byrsonima intermedia	Hexane	1.25	14.1 ± 12.4 (*f)	-3.9 ± 14.5 (ab)
		2.50	9.6 ± 16.3 (*f)	6.9 ± 22.2 (abc)
		3.75	-7.8 ± 11.4 (de)	1.4 ± 14.3 (abc)
		5.00	-21.1 ± 13.2 (*ac)	1.4 ± 14.6 (abc)
	Ethyl Acetate	1.25	0.4 ± 11.1 (e)	6.1 ± 16.7 (abc)
		2.50	-13.4 ± 14.7 (*cd)	13.0 ± 22.2 (c)
		3.75	-20.5 ± 16.2 (*ac)	-6.5 ± 25.8 (a)
		5.00	-24.1 ± 14.5 (*a)	1.0 ± 26.6 (abc)
	Methanol	1.25	-23.9 ± 13.1 (*a)	9.2 ± 19.9 (bc)
		2.50	-61.5 ± 10.6 (*b)	-3.1 ± 28.6 (ab)
		3.75	-58.6 ± 15.4 (*b)	5.6 ± 24.1 (abc)
		5.00	-67.3 ± 9.9 (*b)	3.6 ± 17.3 (abc)
	Negative Control	-	8.47 ± 1.16 cm	2.25 ± 0.34 cm
Moquiniastrum polymorphum	Hexane	1.25	6.4 ± 15.4 (f)	11.3 ± 21.4 (c)
		2.50	-2.6 ± 15.0 (f)	9.6 ± 26.5 (c)
		3.75	-14.9 ± 19.5 (*e)	1.4 ± 26.3 (bc)
		5.00	-18.4 ± 18.4 (*ce)	4.8 ± 29.2 (bc)
	Ethyl Acetate	1.25	-13.8 ± 16.5 (*e)	-10.3 ± 29.6 (ab)
		2.50	-14.1 ± 13.4 (*e)	-10.6 ± 22.8 (ab)
		3.75	-26.9 ± 11.2 (*cd)	-8.0 ± 27.8 (b)
		5.00	-34.6 ± 12.8 (*bd)	-25.6 ± 26.7 (*a)
	Methanol	1.25	-20.1 ± 12.8 (*ce)	-0.7 ± 24.8 (bc)
		2.50	-37.3 ± 11.4 (*b)	12.8 ± 29.0 (c)
		3.75	-39.0 ± 12.8 (*b)	10.9 ± 27.8 (c)
		5.00	-49.1 ± 10.5 (*a)	16.3 ± 20.5 (c)
	Negative Control	-	7.82 ± 1.73 cm	2.08 ± 0.56 cm
Luehea candicans	Hexane	1.25	0.0 ± 12.1 (d)	6.9 ± 21.3 (cde)
		2.50	-1.7 ± 12.8 (d)	11.0 ± 15.4 (de)
		3.75	-0.6 ± 11.8 (d)	3.0 ± 11.5 (cdf)
		5.00	-1.9 ± 11.1 (d)	16.2 ± 18.2 (*e)
	Ethyl Acetate	1.25	-20.8 ± 13.2 (*c)	-13.2 ± 21.4 (*a)
		2.50	-24.2 ± 12.0 (*bc)	1.8 ± 17.9 (bcd)
		3.75	-27.1 ± 12.7 (*bc)	-4.9 ± 21.7 (abf)
		5.00	-30.3 ± 13.2 (*b)	-9.6 ± 21.3 (ab)
	Methanol	1.25	-25.7 ± 15.6 (*bc)	4.8 ± 17.8 (cdef)
		2.50	-29.9 ± 14.4 (*b)	-2.0 ± 18.5 (abc)
		3.75	-43.0 ± 11.3 (*a)	1.0 ± 14.6 (bcd)
		5.00	-44.3 ± 13.0 (*a)	5.3 ± 17.1 (cdef)
	Negative Control	-	9.15 ± 0.92 cm	2.61 ± 0.32 cm
Miconia chamissois	Hexane	1.25	-9.1 ± 14.9 (fg)	-2.2 ± 25.4 (cd)
		2.50	-14.8 ± 14.4 (*efg)	-7.3 ± 21.1 (bcd)
		3.75	-39.2 ± 19.2 (*b)	-27.4 ± 19.0 (*a)
		5.00	-55.5 ± 28.5 (*a)	-25.2 ± 34.7 (*a)
	Ethyl Acetate	1.25	-8.5 ± 12.9 (fg)	6.5 ± 20.1 (d)
		2.50	-6.2 ± 12.6 (g)	6.1 ± 20.3 (d)
		3.75	-5.8 ± 12.1 (g)	6.1 ± 22.7 (d)
		5.00	-16.9 ± 11.7 (*ef)	-7.1 ± 35.9 (bcd)
	Methanol	1.25	-20.1 ± 9.0 (*de)	6.1 ± 22.0 (d)
		2.50	-28.0 ± 10.9 (*cd)	0.7 ± 25.6 (cd)
		3.75	-33.3 ± 16.2 (*bc)	-15.1 ± 23.2 (abc)
		5.00	-29.6 ± 13.0 (*bcd)	-20.9 ± 23.4 (*ab)
	Negative Control	-	9.52 ± 1.69 cm	2.21 ± 0.47 cm
Qualea cordata	Hexane	1.25	14.7 ± 13.2 (*i)	-4.6 ± 17.7 (-)
		2.50	10.4 ± 10.6 (hi)	7.9 ± 17.9 (-)
		3.75	0,2 ± 13.7 (gh)	5.6 ± 14.5 (-)
		5.00	-7.9 ± 12.7 (fg)	12.7 ± 17.3 (*-)
	Ethyl Acetate	1.25	-8.1 ± 17.7 (fg)	-0.7 ± 16.8 (-)
		2.50	-11.3 ± 17.3 (*ef)	2.2 ± 21.8 (-)
		3.75	-27.6 ± 24.5 (*cd)	2.3 ± 20.1 (-)
		5.00	-35.1 ± 25.3 (*c)	4.8 ± 21.1 (-)
	Methanol	1.25	-21.4 ± 9.3 (*de)	-19.0 ± 17.5 (*-)
		2.50	-35.6 ± 12.5 (*c)	-6.4 ± 17.0 (-)
		3.75	-48.5 ± 15.3 (*b)	-9.2 ± 15.8 (-)
		5.00	-63.3 ± 11.9 (*a)	-1.8 ± 22.3 (-)
	Negative Control	-	8.86 ± 1.09 cm	2.51 ± 0.39 cm

Note: *Significant difference between the negative control and the treated according to ANOVA and Dunnett's test. Significant differences among treatments are indicated by different letters according to factorial ANOVA and Tukey's test for the interaction among extracts and concentrations ($p \leq 0.05$). (-) No interaction among extracts and concentrations. All statistical tests were performed on absolute values.

Table 2. Preliminary phytochemical analysis of leaf extracts of medicinal plants of Cerrado.

Extract	Chemical test							
	As	AQs	CMs	STs	TRPs	FLVs	SPs	Ts
B. intermedia								
Hexane	+++	-	-	-	-	-	-	-
Ethyl acetate	+	+	-	-	+	-	++	++
Methanol	+	+	-	-	+++	-	+++	++
M. polymorphum								
Hexane	+++	-	-	-	-	-	+	-
Ethyl acetate	++	-	-	-	-	-	++	-
Methanol	-	-	-	++	-	+	-	-
L. candicans								
Hexane	+++	-	-	-	-	-	+	-
Ethyl acetate	+	-	-	++	-	-	-	-
Methanol	-	-	+	-	+++	+	++	+
M. chamissois								
Hexane	+++	-	-	-	-	-	-	-
Ethyl acetate	++	+	-	-	+	-	-	-
Methanol	++	+++	-	++	-	-	-	++
Q. cordata								
Hexane	+++	-	-	-	-	-	-	-
Ethyl acetate	+++	+++	-	++	-	-	-	++
Methanol	+++	+++	-	-	+	-	+	++

Note: (-): Negative test; (+): Weak positive test; (++): Positive test and (+++): Test strongly positive. The terms used were: As: Alkaloids (R. Dragendorff); AQs: Anthraquinones; CMs: Coumarins; STs: Steroids; TRPs: Triterpenoids; FLVs: Flavonoids; SPs: Saponins; Ts: Tannins.

the phytotoxicity of plant extracts. This trend of greater sensitivity in the roots has been widely reported in the literature (Chon et al., 2000; Habermann et al., 2016; Anwar et al., 2020; Lima et al., 2021). This is likely because the roots are in direct contact with the extracts and are therefore exposed to higher doses of chemical compounds (Tanveer et al., 2012; Alves et al., 2022).

Differences in the effectiveness of plant extracts may be related to the fact that specific compounds are extracted in greater amounts based on their affinity with different solvents (Luthria and Mukhopadhyay, 2006; Corrêa et al., 2008). Thus, the effectiveness of extracting chemical compounds from plant materials and the phytotoxicity of extracts depends on the different solvents used for extraction (Luthria et al., 2007; Ladhari et al., 2020). In the present study, we observed that the phytotoxic effects of organic extracts that had already undergone the extraction process in less-polar organic solvents were more effective than those only extracted in less-polar solvents. For example, the methanolic extracts of *B. intermedia*, *M. polymorpha*, *L. candicans*, and *Q. cordata* showed higher phytotoxicity than the hexane and ethyl acetate extracts. Removing non-active chemical compounds may increase the concentration of active chemical compounds in the final extract and accentuate its phytotoxicity (Athmouni et al., 2015). However, this pattern was not observed for *M. chamissois*, the hexane extract that showed higher phytotoxicity than more polar solvents. Therefore, our results show that phytotoxicity varies according to the solvent used and that using solvents with different polarities can result in different extraction yields of bioactive compounds and different phytotoxic effects (Athmouni et al., 2015).

The hexane extract of *M. chamissois* showed a strong positive result for alkaloids, while other classes of secondary metabolites were not detected, suggesting that these compounds are responsible for the phytotoxic effects observed. This chemical class was also detected in the hexane extract of the other study species; however, the literature provided support for alkaloids presence only for related taxa of *M. moquiniastrum* (Faustino et al., 2017). Nebo et al. (2014) evaluated the phytotoxicity of two alkaloids isolated from *Ruta graveolens* on the growth parameters of four target species. The authors observed that the alkaloids had a potent phytotoxic effect comparable with the effects of a commercial herbicide.

The methanolic extracts of *B. intermedia* and *Q. cordata* exhibited positive results for five chemical classes. Our findings are supported by previous reports, which described some of the same chemical classes in these

species. Specifically, triterpenoids were described for several *Qualea* species (Carnevale Neto et al., 2011), and triterpenoids, saponins, and tannins for *B. intermedia* (Orlandi et al., 2011). Thus, the high phytotoxicity on cucumber root growth may be associated with the presence of these compounds. Matsumoto et al. (2010) tested the effects of *Annona glabra* extracts on some target species and attributed the deleterious effects on plant growth to the action of triterpenes, tannins, and flavonoids. Also, several isolated saponins from different species have been shown to inhibit the growth of target plants (Hernández-Carlos et al., 2011; Scognamiglio et al., 2012). It has been proposed that saponins inhibit plant development by forming high-molecular-weight matrices around the radicle, which prevents water uptake by plants (Waller et al., 1999).

The methanolic extract of *M. polymorphum* tested positive for two chemical classes, one of which is flavonoids. This finding is consistent with a previous report of the presence of flavonoids in this species (Gonçalves et al., 2019; Moreira et al., 2000), confirming our result. Therefore, this chemical class may be related to the phytotoxic effects observed on cucumber growth. A study has shown the phytotoxic potential of flavonoids isolated from species of Rutaceae, which were toxic to *Lepidium sativum* (Nebo et al., 2014).

The constituents of the methanolic extract of *L. candicans* leaves included triterpenoids, and flavonoids, as suggested by Tanaka et al. (2005) for related taxon. These chemical compounds or changes in their concentrations may be related to the high phytotoxicity at the early growth stage of cucumber. In addition, our study suggested the presence of anthraquinones in extracts of *B. intermedia*, saponins in *M. polymorphum*, coumarins, flavonoids, saponins, and tannins in *L. candicans*, anthraquinones, triterpenoids, and tannins in *M. chamissois*, and anthraquinones, saponins, and tannins in *Q. cordata*, as well as steroids for most of the species evaluated. However, there is a lack of studies with unequivocal analytical methods for confirming these substances in these taxa.

The phytotoxicity of the chemical classes found in the present study has been widely reported in the literature. Alkaloids affect the ultrastructure of root apices by promoting organelle disorganization, the occurrence of lipid droplets, and increases in size and number of vacuoles (Liu and Lovett, 1993). This may be due to their ability to interact with lipids, causing disturbances in cell membranes, and negatively affecting root growth (Lebecque et al., 2018). Recently, some authors have shown that the root caps of primary and lateral roots form a cuticle in the early stages of development. This structure is present in the primordia of the lateral roots and, when defective, causes delayed emergence and root deformation (Berhin et al., 2019). Since the cuticle is formed by lipids, alkaloids may impair the integrity of this cuticle, causing a reduction in the number of lateral roots formed, as observed for some of the extracts studied here. Among the phenolic compounds, tannins are responsible for the formation of superoxide anions and hydroxyl radicals by donating electrons to molecular oxygen, which alters the permeability of the membrane and cellular components, such as DNA and proteins. Flavonoids have been reported to trigger the accumulation of reactive oxygen species, leading to the calcium ion signaling cascade and the death of the root system (Martins et al., 2021). Additionally, these compounds can alter the transport of minerals in plants, besides altering the structure of chloroplasts and mitochondria.

The present study demonstrates the phytotoxicity of different concentrations of organic extracts from leaves of *B. intermedia*, *M. polymorphum*, *L. candicans*, *M. chamissois*, and *Q. cordata*. The phytotoxic effect was mainly characterized by the inhibition of root development. The extracts with the greatest phytotoxicity were the methanol extracts of *B. intermedia*, *M. polymorphum*, *L. candicans*, and *Q. cordata* and the hexane extract of *M. chamissois.* We also provide an opportunity to characterize and purify the chemical compounds responsible for the phytotoxic effects of the most active extracts. Identifying these chemical compounds can contribute to a better understanding of the phytotoxicity of the Cerrado species and provide opportunities for environmentally friendly weed management.

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References

ALGANDABY, M.M. and EL-DARIER, S.M., 2018. Management of the noxious weed; *Medicago polymorpha* L. via allelopathy of some medicinal plants from Taif region, Saudi Arabia. *Saudi Journal of Biological Sciences*, vol. 25, no. 7, pp. 1339-1347. http://dx.doi.org/10.1016/j.sjbs.2016.02.013. PMid:30505179.

ALVES, T.A., SPADETO, M.S., VASCONCELOS, L.C., SOUZA, J.R.C.L., MENINI, L., FERREIRA, M.F.S. and PRAÇA-FONTES, M.M., 2022. Phytotoxicity and cytogenetic action mechanism of leaf extracts of *Psidium cattleyanum* Sabine in plant bioassays. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, e260985. http://dx.doi.org/10.1590/1519-6984.260985. PMid:35674589.

ANWAR, T., QURESHI, H., PARVEEN, N., BASHIR, R., QAISAR, U., MUNAZIR, M., YASMIN, S., BASIT, Z., MAHMOOD, R.T., NAYYAR, B.G., KHAN, S., KHAN, S.A., QURESHI, M.M. and WALI, M., 2020. Evaluation of bioherbicidal potential of *Carica papaya* leaves. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 80, no. 3, pp. 565-573. http://dx.doi.org/10.1590/1519-6984.216359. PMid:31644650.

ATHMOUNI, K., BELGHITH, T., BELLASSOUAD, K., EL FEKI, A. and AYADI, H., 2015. Effect of solvent polarity on the content of biomolecules and antioxidant activity of *Thapsia garganica* (Apiacea). *Algerian Journal of Natural Products*, vol. 3, no. 3, pp. 194-208. http://dx.doi.org/10.1025/ajnp.2015.3.3.194.

BERHIN, A., DE BELLIS, D., FRANKE, R.B., BUONO, R.A., NOWACK, M.K. and NAWRATH, C., 2019. The root cap cuticle: a cell wall structure for seedling establishment and lateral root formation. *Cell*, vol. 176, no. 6, pp. 1367-1378.e8. http://dx.doi.org/10.1016/j.cell.2019.01.005. PMid:30773319.

CARLI, C.B.A., QUILLES, M.B. and CARLOS, I.Z., 2013. Natural products with activity against multidrug-resistant tumor cells. In: M.K. RAI and K.V. KON, eds. *Fighting multidrug resistance with herbal*

extracts, essential oils and their components. London: Academic Press, pp. 237-244. http://dx.doi.org/10.1016/B978-0-12-398539-2.00016-1.

CARNEVALE NETO, F., PILON, A.C., SILVA, D.H.S., BOLZANI, V.S. and CASTRO-GAMBOA, I., 2011. Vochysiaceae: secondary metabolites, ethnopharmacology and pharmacological potential. *Phytochemistry Reviews*, vol. 10, no. 3, pp. 413-429. http://dx.doi.org/10.1007/s11101-011-9213-5.

CARNEVALE NETO, F., SIQUITELLI, C.D., PILON, A.C., SILVA, D.H.S., BOLZANI, V.S. and CASTRO-GAMBOA, I., 2013. Dereplication of phenolic derivates of *Qualea grandiflora* and *Qualea cordara* (Vochysiaceae) using liquid chromatography coupled with ESI-QToF-MS/MS. *Journal of the Brazilian Chemical Society*, vol. 24, no. 5, pp. 758-764. http://dx.doi.org/10.5935/0103-5053.20130098.

CECCHIN, K., FAVARETTO, A., SCHEFFER-BASSO, S.M., BERTOL, C.D. and CHINI, S.O., 2017. Allelopathy and allelochemicals of *Eragrostis plana* (Poaceae) and its relation with phenology and nitrogen fertilization. *Planta Daninha*, vol. 35, e017157907. http://dx.doi.org/10.1590/s0100-83582017350100028.

CHON, S.U., COUTTS, J.H. and NELSON, C.J., 2000. Effects of light, growth media and seedling orientation on bioassays of alfalfa autotoxicity. *Agronomy Journal*, vol. 92, no. 4, pp. 715-720. http://dx.doi.org/10.2134/agronj2000.924715x.

CHUNG, I.M., AHN, J.K. and YUN, S.J., 2001. Assessment of allelopathic potential of barnyard grass (*Echinochloa crus-galli*) on rice (*Oryza sativa* L.) cultivars. *Crop Protection*, vol. 20, no. 10, pp. 921-928. http://dx.doi.org/10.1016/S0261-2194(01)00046-1.

CORRÊA, L.R., SOARES, G.L.G. and FETT-NETO, A.G., 2008. Allelopathic potential of *Psychotria leiocarpa*, a dominant understorey species of subtropical forests. *South African Journal of Botany*, vol. 74, no. 4, pp. 583-590. http://dx.doi.org/10.1016/j.sajb.2008.02.006.

DAYAN, F.E., CANTRELL, C.L. and DUKE, S.O., 2009. Natural products in crop protection. *Bioorganic & Medicinal Chemistry*, vol. 17, no. 12, pp. 4022-4034. http://dx.doi.org/10.1016/j.bmc.2009.01.046. PMid:19216080.

DUKE, S.O., DAYAN, F.E., ROMAGNI, J.G. and RIMANDO, A.M., 2000. Natural products as sources of herbicides: current status and future trends. *Weed Research*, vol. 40, no. 1, pp. 99-111. http://dx.doi.org/10.1046/j.1365-3180.2000.00161.x.

EBERLEIN, C.V., 1987. Germination of *Sorghum almum* seeds and longevity in soil. *Weed Science*, vol. 35, no. 6, pp. 796-801. http://dx.doi.org/10.1017/S0043174500079364.

FAUSTINO, M.V., SECA, A.M.L., SILVEIRA, P., SILVA, A.M.S. and PINTO, D.C.G.A., 2017. Gas chromatography–mass spectrometry profile of four *Calendula* L. taxa: a comparative analysis. *Industrial Crops and Products*, vol. 104, pp. 91-98. http://dx.doi.org/10.1016/j.indcrop.2017.04.029.

FERREIRA, A.G. and AQUILA, M.E.A., 2000. Alelopatia: uma área emergente da ecofisiologia. *Revista Brasileira de Fisiologia Vegetal*, vol. 12, pp. 175-204.

GONÇALVES, V.M., MOREIRA, A.S. and OLIVEIRA, P.D., 2019. Genus *Moquiniastrum* (Asteraceae): overview of chemical and bioactivity studies. *Current Bioactive Compounds*, vol. 15, no. 4, pp. 377-398. http://dx.doi.org/10.2174/1573407214666180524094146.

GUILHON-SIMPLICIO, F. and PEREIRA, M.M., 2011. Aspectos químicos e farmacológicos de *Byrsonima* (Malpighiaceae). *Quimica Nova*, vol. 34, no. 6, pp. 1032-1041. http://dx.doi.org/10.1590/S0100-40422011000600021.

HABERMANN, E., PONTES, F.C., PEREIRA, V.C., IMATOMI, M. and GUALTIERI, S.C.J., 2016. Phytotoxic potential of young leaves from *Blepharocalyx salicifolius* (Kunth) O. Berg (Myrtaceae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 76, no. 2, pp. 531-538. http://dx.doi.org/10.1590/1519-6984.24114. PMid:26959947.

HERNÁNDEZ-CARLOS, B., GONZÁLEZ-COLOMA, A., OROZCO-VALENCIA, A.U., RAMÍREZ-MARES, M.V., ANDRÉS-YEVES, M.F. and JOSEPH-NATHAN, P., 2011. Bioactive saponins from *Microsechium helleri* and *Sicyos bulbosus*. *Phytochemistry*, vol. 72, no. 8, pp. 743-751. http://dx.doi.org/10.1016/j.phytochem.2011.02.022. PMid:21439597.

LADHARI, A., GAALICHE, B., ZARRELLI, A., GHANNEM, M. and MIMOUN, M.B., 2020. Allelopathic potential and phenolic allelochemicals discrepancies in *Ficus carica* L. cultivars. *South African Journal of Botany*, vol. 130, pp. 30-44. http://dx.doi.org/10.1016/j.sajb.2019.11.026.

LEBECQUE, S., CROWET, J.M., LINS, L., DELORY, B.M., DU JARDIN, P., FAUCONNIER, M.L. and DELEU, M., 2018. Interaction between the barley allelochemical compounds gramine and hordenine and artificial lipid bilayers mimicking the plant plasma membrane. *Scientific Reports*, vol. 8, no. 9784, pp. 9784. http://dx.doi.org/10.1038/s41598-018-28040-6. PMid:29955111.

LIMA, L.M., PEDROZA, L.S., OSÓRIO, M.I.C., SOUZA, J.C. and NUNEZ, C.V., 2021. Phytotoxicity of plant extracts of *Vismia japurensis* cultivated in vivo and in vitro. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e235475. http://dx.doi.org/10.1590/1519-6984.235475. PMid:34105661.

LIU, D.L. and LOVETT, J.V., 1993. Biologically active secondary metabolites of barley. II. Phytotoxicity of barley allelochemicals. *Journal of Chemical Ecology*, vol. 19, no. 10, pp. 2231-2244. http://dx.doi.org/10.1007/BF00979660. PMid:24248572.

LUTHRIA, D.L. and MUKHOPADHYAY, S., 2006. Influence of sample preparation on assay of phenolic acids from eggplant. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 1, pp. 41-47. http://dx.doi.org/10.1021/jf0522457. PMid:16390175.

LUTHRIA, D.L., BISWAS, R. and NATARAJAN, S., 2007. Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chemistry*, vol. 105, no. 1, pp. 325-333. http://dx.doi.org/10.1016/j.foodchem.2006.11.047.

MARTINS, S.A., SANTOS, R.C., RAMOS, A.R., FIGUEIREDO, P.L.B., SILVA, C.R.C. and SILVA, J.K.R., 2021. Allelopathic potential and phytochemical screening of *Piper divaricatum* extracts on germination and growth of indicator plant (*Lactuca sativa*). *South African Journal of Botany*, vol. 138, pp. 495-499. http://dx.doi.org/10.1016/j.sajb.2021.01.014.

MATOS, F.J.A., 1997. *Introdução a fitoquímica experimental*. Fortaleza: Edições UFC.

MATSUMOTO, R.S., RIBEIRO, J.P.N., TAKAO, L.K. and LIMA, M.I.S., 2010. Allelopathic potential of leaf extract of *Annona glabra* L. (Annonaceae). *Acta Botanica Brasílica*, vol. 24, no. 3, pp. 631-635. http://dx.doi.org/10.1590/S0102-33062010000300005.

MICHELIN, D.C., SANNOMIYA, M., FIGUEIREDO, M.E., RINALDO, D., SANTOS, L.C., SOUZA-BRITO, A.R.M., VILEGAS, W. and SALGADO, H.R.N., 2008. Antimicrobial activity of *Byrsonima* species (Malpighiaceae). *Revista Brasileira de Farmacognosia*, vol. 18, pp. 690-695. http://dx.doi.org/10.1590/S0102-695X2008000500009.

MOREIRA, A.S., SPITZER, V., SCHAPOVAL, E.E. and SCHENKEL, E.P., 2000. Antiinflammatory activity of extracts and fractions from the leaves of *Gochnatia polymorpha*. *Phytotherapy Research*, vol. 14, no. 8, pp. 638-640. http://dx.doi.org/10.1002/1099-1573(200012)14:8<638::AID-PTR681>3.0.CO;2-Q. PMid:11114003.

MOREIRA, L.Q., VILELA, F.C., ORLANDI, L., DIAS, D.F., SANTOS, A.L.A., SILVA, M.A., PAIVA, R., ALVES-DA-SILVA, G. and GIUSTI-PAIVA, A., 2011. Anti-inflammatory effect of extract and fractions from

the leaves of *Byrsonima intermedia* A. Juss. in rats. *Journal of Ethnopharmacology*, vol. 138, no. 2, pp. 610-615. http://dx.doi.org/10.1016/j.jep.2011.10.006. PMid:22008877.

NEBO, L., VARELA, R.M., MOLINILLO, J.M.G., SAMPAIO, O.M., SEVERINO, V.G.P., CAZAL, C.M., FERNANDES, M.F.G., FERNANDES, J.B. and MACÍAS, F.A., 2014. Phytotoxicity of alkaloids, coumarins and flavonoids isolated from 11 species belonging to the Rutaceae and Meliaceae families. *Phytochemistry Letters*, vol. 8, pp. 444-447. http://dx.doi.org/10.1016/j.phytol.2014.02.010.

ORLANDI, L., VILELA, F.C., SANTA-CECÍLIA, F.V., DIAS, D.F., ALVES-DA-SILVA, G. and GIUSTI-PAIVA, A., 2011. Anti-inflammatory and antinociceptive effects of the stem bark of *Byrsonima intermedia* A. Juss. *Journal of Ethnopharmacology*, vol. 137, no. 3, pp. 1469-1476. http://dx.doi.org/10.1016/j.jep.2011.08.032. PMid:21878381.

PETROVA, S.T., VALCHEVA, E.G. and VELCHEVA, I.G., 2015. A case study of allelopathic effect on weeds in wheat. *Ecologia Balkanica*, vol. 7, no. 1, pp. 121-129.

PINTO, G.F.S. and KOLB, R.M., 2016. Seasonality affects phytotoxic potential of five native species of Neotropical savanna. *Botany*, vol. 94, no. 2, pp. 1-9. http://dx.doi.org/10.1139/cjb-2015-0124.

QASEM, J.R., 2002. Allelopathic effects of selected medicinal plants on *Amaranthus retroflexus* and *Chenopodium murale*. *Allelopathy Journal*, vol. 10, no. 2, pp. 105-122.

REDDY, M.V., UMAJYOTHI, K., REDDY, P.S.S. and SASIKALA, K., 2019. Effect of pre and post emergence herbicides on nutrient removal and uptake in onion Cv. N - 53. *International Journal of Chemical Studies*, vol. 7, no. 5, pp. 1809-1812.

REIGOSA, M., GOMES, A.S., FERREIRA, A.G. and BORGHETTI, F., 2013. Allelopathic research in Brazil. *Acta Botanica Brasílica*, vol. 27, no. 4, pp. 629-646. http://dx.doi.org/10.1590/S0102-33062013000400001.

RICE, E.L., 1979. Allelopathy–an update. *Botanical Review*, vol. 45, no. 1, pp. 15-109. http://dx.doi.org/10.1007/BF02869951.

ROJAS DE ARIAS, A., FERRO, E., INCHAUSTI, A., ASCURRA, M., ACOSTA, N., RODRIGUEZ, E. and FOURNET, A., 1995. Mutagenicity, insecticidal and trypanocidal activity of some Paraguayan Asteraceae. *Journal of Ethnopharmacology*, vol. 45, no. 1, pp. 35-41. http://dx.doi.org/10.1016/0378-8741(94)01193-4. PMid:7739225.

ROSA, M.N., SILVA, L.R.V., LONGATO, G.B., EVANGELISTA, A.F., GOMES, I.N.F., ALVES, A.L.V., OLIVEIRA, B.G., PINTO, F.E., ROMÃO, W., REZENDE, A.R., ARAÚJO, A.A.C., OLIVEIRA, L.S.F.M., SOUZA, A.A.M., OLIVEIRA, S.C., RIBEIRO, R.I.M.A., SILVA, V.A.O. and REIS, R.M., 2021. Bioprospecting of natural compounds from Brazilian Cerrado biome plants in human cervical cancer cell lines. *International Journal of Molecular Sciences*, vol. 22, no. 7, pp. 3383. http://dx.doi.org/10.3390/ijms22073383. PMid:33806119.

SCOGNAMIGLIO, M., D'ABROSCA, B., FIUMANO, V., CHAMBERY, A., SEVERINO, V., TSAFANTAKIS, N., PACIFICO, S., ESPOSITO, A. and FIORENTINO, A., 2012. Oleanane saponins from *Bellis sylvestris* Cyr. and evaluation of their phytotoxicity on *Aegilops geniculata* Roth. *Phytochemistry*, vol. 84, pp. 125-134. http://dx.doi.org/10.1016/j.phytochem.2012.08.006. PMid:22959224.

SILVA, A.G., SILVA, V.A.O., OLIVEIRA, R.J.S., REZENDE, A.R., CHAGAS, R.C.R., PIMENTA, L.P.S., ROMÃO, W., SANTOS, H.B., THOMÉ, R.G., REIS, R.M. and RIBEIRO, R.I.M.A., 2020. Matteucinol, isolated from *Miconia chamissois*, induces apoptosis in human glioblastoma lines via the intrinsic pathway and inhibits angiogenesis and tumor growth in vivo. Invest. *Investigational New Drugs*, vol. 38, no. 4, pp. 1044-1055. http://dx.doi.org/10.1007/s10637-019-00878-1. PMid:31781904.

SILVA, D.A., ALVES, V.G., FRANCO, D.M.M., RIBEIRO, L.C., SOUZA, M.C., KATO, L., CARVALHO, J.E., KOHN, L.K., OLIVEIRA, C.M.A. and SILVA, C.C., 2012. Antiproliferative activity of *Luehea candicans* Mart. et Zucc. (Tiliaceae). *Natural Product Research*, vol. 26, no. 4, pp. 364-369. http://dx.doi.org/10.1080/14786411003752102. PMid:21432719.

SIMÕES, C.M., FALKENBERG, M., MENTZ, L.A., SCHENKEL, E.P., AMOROS, M. and GIRRE, L., 1999. Antiviral activity of south Brazilian medicinal plant extracts. *Phytomedicine*, vol. 6, no. 3, pp. 205-214. http://dx.doi.org/10.1016/S0944-7113(99)80010-5. PMid:10439486.

TANAKA, J.C.A., SILVA, C.C., DIAS FILHO, B.P., NAKAMURA, C.V., CARVALHO, J.E. and FOGLIO, M.A., 2005. Constituintes químicos de *Luehea divaricata* Mart. (Tiliaceae). *Química Nova*, vol. 28, no. 5, pp. 834-837. http://dx.doi.org/10.1590/S0100-40422005000500020.

TANVEER, A., JABBAR, M.K., KAHLIQ, A., MATLOOB, A., ABBAS, R.N. and JAVAID, M.M., 2012. Allelopathic effects of aqueous and organic fractions of *Euphorbia dracunculoides* Lam. on germination and seedling growth of chickpea and wheat. *Chilean Journal of Agricultural Research*, vol. 72, no. 4, pp. 495-501. http://dx.doi.org/10.4067/S0718-58392012000400006.

TEERARAK, M., LAOSINWATTANA, C. and CHAROENYING, P., 2010. Evaluation of allelopathic, decomposition and cytogenetic activities of *Jasminum officinale* L. f. var. *grandiflorum* (L.) Kob. on bioassay plants. *Bioresource Technology*, vol. 101, no. 14, pp. 5677-5684. http://dx.doi.org/10.1016/j.biortech.2010.02.038. PMid:20199861.

VILLELA, F.A., DONI FILHO, L. and SEQUEIRA, E.L., 1991. Tabela de potencial osmótico em função da concentração de polietileno glicol 6.000 e da temperatura. *Pesquisa Agropecuária Brasileira*, vol. 26, no. 11-12, pp. 1957-1968.

WALLER, G.R., YANG, C.F., CHEN, L.F., SU, C.H., LIOU, R.M., WU, S.C., YOUNG, C.C., LEE, M.R., LEE, J.S., CHENG, C.S., CHOU, C.H. and KIM, D., 1999. Saponins produced during the life cycle of mungbeans and their role as allelochemicals. *Studies in Plant Science*, vol. 6, pp. 105-130. http://dx.doi.org/10.1016/S0928-3420(99)80015-9.