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PHYTOCHEMICAL PROFILE OF PASTURE WEEDS FROM THE BRAZILIAN CERRADO

Perfil Fitoquímico de Plantas Daninhas de Pastagens do Cerrado Brasileiro

ABSTRACT - The great diversity of plant species in pastures of the Brazilian Cerrado with distinct ecophysiological characteristics indicates the possibility of finding, in the cellular content of this flora, chemical compounds with potential for use in agriculture and human and animal health. Three steps are necessary to prove this hypothesis: phytochemical prospecting, characterization of secondary metabolites, and studies on the biological activities of these metabolites present in these plants. The chemical profile of secondary metabolites present in five species of the Brazilian Cerrado (*Davilla elliptica*, *Remijia ferruginea*, *Luehea paniculata*, *Anacardium occidentale*, and *Acosmium dasycarpum*) was traced in this research. These plant species were collected in pasture areas of Felício dos Santos, Minas Gerais. The samples were dried and submitted to two types of extract (ethanolic and hexanic). By using specific chemical reactions, the presence of coumarins, triterpenes/steroids, and anthracenoides was observed in the hexanic extracts, while the presence of alkaloids, triterpenes/steroids, flavonoids, tannins, reducing compounds, and anthocyanins was observed in the ethanolic extracts. The species presented diverse classes of compounds. However, triterpenes/steroids, tannins, reducing compounds and anthocyanins were found in all species, being the use of the solvent ethanol the most efficient in extracting the compounds. The species *D. elliptica* presented the highest number of classes of compounds. The widespread application in folk medicine justifies further studies on the biological activity of different metabolites in agriculture and health areas.

Keywords: secondary metabolites, *Davilla elliptica*, *Remijia ferruginea*, *Luehea paniculata*, *Anacardium occidentale*, *Acosmium dasycarpum*, weed, allelopathy.

RESUMO - A grande diversidade de espécies vegetais nas pastagens do cerrado brasileiro com características ecofisiológicas diferenciadas indica a possibilidade de encontrar no conteúdo celular dessa flora compostos químicos com potencial para uso na agricultura, saúde humana e animal. Para comprovação dessa hipótese, serão necessárias três etapas: a prospecção fitoquímica, a caracterização dos metabólitos secundários e os estudos sobre as atividades biológicas desses metabólitos presentes nessas plantas. Nesta pesquisa foi traçado o perfil químico dos metabólitos secundários presentes em cinco espécies do cerrado brasileiro (*Davilla elliptica*, *Remijia ferruginea*, *Luehea paniculata*, *Anacardium occidentale* e *Acosmium dasycarpum*). A coleta das plantas foi realizada em áreas de pastagem do município de Felício dos Santos, Minas Gerais. As amostras foram secas e submetidas a dois tipos de extrato (etanólico e hexânico). Empregando reações químicas específicas, constatou-se nos extratos hexânicos a presença de cumarinas, triterpenos/esteroides, e antracenosídeos e, nos extratos etanólicos, de alcaloides,

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triterpenos/esteroides, flavonoides, taninos, compostos redutores e antocianinas. As espécies apresentaram diversificadas classes de compostos. Entretanto, em todas as espécies foram encontrados triterpenos/esteroides, taninos, compostos redutores e antocianinas, sendo o emprego do solvente etanol o mais eficiente na extração dos compostos. A espécie D. elliptica foi a que apresentou o maior número de classes de compostos. Considerando a sua ampla aplicação na medicina popular, justificam-se novos estudos sobre atividade biológica dos diferentes metabólitos nas áreas agrícola e da saúde.

Palavras-chave: metabólitos secundários, *Davilla elliptica*, *Remijia ferrugínea*, *Luehea paniculata*, *Anacardium occidentale*, *Acosmium dasycarpum*, planta daninha, alelopatia.

INTRODUCTION

The Cerrado biome is the second largest Brazilian plant formation, being quite heterogeneous and peculiar, with a valuable chemical arsenal (Maffei et al., 2011; Maracahipes et al., 2011). Among the agricultural activities developed in this biome, livestock farming currently occupies the largest area (Rodrigues and Miziara, 2008). One of the most important factors for the success of this activity is the quality of pastures, which is often affected by the occurrence of weeds, especially those toxic to animals (Jakelaitis et al., 2010). These plants compete with forage plants for light, water, nutrients, and physical space (Silva and Saliba, 2007), scratch animals, devaluing the leather, and are also responsible, when toxic, for the mortality of some animals (Antoniassi et al., 2007).

Some species are commonly found in pastures of the Cerrado, such as *Davilla elliptica* (Rocha Filho and Lomônaco, 2006), *Remijia ferrugínea* (Botsaris, 2007; Cosenza et al., 2013), *Luehea paniculata*, *Anacardium occidentale* (Neri et al., 2007; Rocha et al., 2011), and *Acosmium dasycarpum* (Lenza and Klink, 2006; Moreno et al., 2008). These species can be sources of secondary metabolites formed by different biosynthetic pathways, which produce molecules with a diversity of skeletons and functional groups, such as fatty acids and their esters, hydrocarbons, alcohols, aldehydes and ketones, acetylenic compounds, alkaloids, phenolic compounds, and coumarins. In recent years, the economic interest in such compounds has increased considerably in different areas of study, such as agrochemical, medicinal, biochemistry, and botany.

Secondary metabolites compose substances that have diverse chemical structures and biological properties (Granato et al., 2013). These compounds represent a chemical interface between the plant and the surrounding environment and their synthesis is directly affected by environmental conditions (Gobbo Neto and Lopes, 2007; Sampaio et al., 2016). They are synthesized by plants and released into the environment via root exudates in the soil or via plant shoot, which may volatilize or leach, leading to beneficial or deleterious effects on other plants or microorganisms (Ahemada and Kibret, 2014; Sadia et al., 2015).

Plants holding bioactive compounds used for medicinal purposes are also sources of active principles of interest in agriculture. As an example, some species with inhibitory allelopathic activity can be used as efficient natural herbicides, controlling weeds, and serve as an indication for possible sources of new compounds with pesticide action, contributing to increasing the productivity and the healthy permanence of cultivars, making the cultivation area more balanced (Silva, 2012).

In recent years, there have been major changes in weed management, with the increasing adoption of herbicides and, in many cases, the use of the same molecule repeatedly in one area. This was mainly due to the spread of no-tillage and, more recently, the use of transgenic crops (Santos et al., 2006; Gomes and Christoffoleti, 2008). This fact led to an increase in selection pressure on plant populations, leading to increasingly frequent cases of herbicide-resistant weed biotypes (Beckie, 2011; Green and Owen, 2011), as well as an increased contamination of water and soil (Cao et al., 2013, Pereira et al., 2016). Therefore, research is increasingly needed to discover new compounds with herbicide potential.

Phytochemical prospecting is the first step in studies on secondary metabolites present in plants and their biological activities. The aim of this research was to trace the chemical profile

of five species found in pasture areas of the Brazilian Cerrado in order to characterize their secondary metabolites and hence contribute to the discovery of possible actions of human interest.

MATERIAL AND METHODS

Collection and identification of the plant

The species were collected in the morning in Felício dos Santos, Minas Gerais, in March 2013 (Table 1 and Figure 1). The taxonomic identification of this plant material was made by a botanist of the Department of Biological Sciences of UFVJM (Universidade Federal dos Vales do Jequitinhonha e Mucuri), and the voucher specimen of each species was deposited in the DIA herbarium belonging to the UFVJM.

Preparation of crude extracts

The aerial structures of the used species (Table 1) were dried from light at ambient temperature until constant weight and ground in a knife mill. Subsequently, crude extracts were obtained from the ground material (50 g) by exhaustive extraction with ethanol, followed by simple filtration using qualitative filter paper with a diameter of 12.5 cm. The ethanolic extract (300 mL) was extracted with hexane (3 x 50 mL). The hexanic fractions were pooled and, separately, the ethanolic and hexanic extracts were concentrated in a rotary evaporator at 40 °C under reduced pressure to obtain the ethanolic (EE) and hexane extract (HE).

Table 1 - Taxonomic and popular identification and used structures of each species under study

Scientific name	Popular name*	Used part
<i>Davilla elliptica</i> A. St.-Hil.	Cipó-caboclo	Leaf
<i>Remijia ferruginea</i> DC.	Quina-de-vara	Sprout/stem
<i>Luehea paniculata</i> Mart	Açoita-cavalo	Bark
<i>Anacardium occidentale</i> L.	Cajuzinho-do-campo	Leaf
<i>Acosmium dasycarpum</i> (Vogel) Yakovlev	Unha-d'anta	Bark

* Popular names were provided by residents of the regional community of Felício dos Santos, Minas Gerais.

Phytochemical prospecting

The main classes of secondary metabolites were identified using a methodology proposed by Matos (1988). Specific chemical reactions were used to determine the presence of coumarins, alkaloids, triterpenes/steroids, flavonoids, and anthracenosides the hexanic extracts, as well as coumarins, triterpenes/steroids, flavonoids, tannins, anthracenosides, reducing compounds, and anthocyanins in the ethanolic extracts.

Reagents for chemical tests

Liebermann-Burchard reagent

The amount of 1 mL acetic anhydride was added to a tube containing 1 mL CHCl₃ under stirring. Thereafter, three to four drops of concentrated sulfuric acid were slowly added (Matos, 1988).

Mayer reactive

Solution A: 1.4 g mercury chloride in 60 mL distilled water.

Solution B: 7.0 g potassium iodide in 20 mL distilled water.



Source: <http://www.tropicos.org/>; <http://floradobrasil.jbrj.gov.br/>.

Figure 1 - Species used to perform the tests.

Both solutions were mixed, stirred, and filtered and the volume was made up to 10 mL with distilled water.

Fehling reagent

Solution A: 34.7 g cupric sulfate in 500 mL distilled water.

Solution B: 173.0 g sodium and potassium bitartrate and 125.0 g potassium hydroxide in 500 mL distilled water.

Solutions A and B were mixed.

Tests performed for phytochemical screening

Test for alkaloids

Part of the extracts from the assessed plants was dissolved in 20 mL 10% HCl. The obtained solutions were divided into fractions A (control) and B. The Mayer reactive was added to tube B.

In the positive test, there is an alteration in the solution, with the formation of a precipitate, which indicates the presence of alkaloids.

Test for triterpenes/steroids

The sample was solubilized in 1 mL CHCl_3 and 1 mL acetic anhydride was added under stirring. Subsequently, three to four drops of concentrated sulfuric acid were slowly added. This test is used for detecting substances containing steroidal or triterpene core. In the positive test for pentacyclic triterpenes, the appearance of permanent violet or blue color is observed. For steroids, the indicative color is green.

Test for tannins

Part of the extracts was solubilized in distilled water and drops of a 1% FeCl_3 solution were added. The appearance of the blue coloration indicates the presence of hydrolyzable or gallic tannins, while the green coloration indicates the presence of condensed or catechin tannins.

Test for flavonoids

Part of the extracts was dissolved in 2 mL CH_3OH and metallic magnesium and 1 mL concentrated HCl were added. Twenty minutes were waited. In the positive test, the appearance of reddish coloration is an indication of the presence of flavonoids.

Test for reducing compounds

Part of the extracts was dissolved in 5 mL ethanol, 2 mL distilled water, and 5 mL Fehling reagent. It was heated in a water bath for 30 minutes. In the positive test, the formation of a brown precipitate is an indication of the presence of reducing compounds.

Test for anthracenosides

Part of the extracts was solubilized in 25 mL ethanol. The amount of 10 mL 20% HCl was added to the obtained solutions, followed by reflux for 30 minutes. Then, 10 mL distilled water was added followed by evaporation to approximately 15 mL. It was extracted with 30 mL CHCl_3 twice. The obtained chloroformic phase was evaporated under reduced pressure to obtain a residue, which was dissolved in 3 mL 25% NH_4OH . In the positive test, the development of a red coloration is observed, which indicates the presence of anthracenosides.

Test for anthocyanins

Part of the extracts was solubilized in 25 mL ethanol. Subsequently, 10 mL 20% HCl was added to the obtained solution, followed by reflux for 30 minutes. Then, 10 mL distilled water was added and evaporated to approximately 15 mL. It was extracted with 30 mL CHCl_3 twice. The pH of the acid aqueous phase was raised to approximately 9. The positive test shows the development of a brownish-green or blue coloration, indicative of the presence of anthocyanins.

RESULTS AND DISCUSSION

The results of phytochemical tests of ethanolic and hexanic extracts are shown in Tables 2 and 3. The confirmation of reactions of phytochemical characterization of the extracts occurred by the following responses: alkaloids due to a precipitate formation; pentacyclic triterpenes due to the appearance of permanent violet or blue color; steroids due to a green coloration; hydrolysable or gallic tannins due to a blue coloration; condensed or catechin tannins due to the appearance of green coloration; flavonoids due to the appearance of a reddish color;

Table 2 - Phytochemical prospecting of hexanic extracts of the studied species for the following compounds: coumarins (A), alkaloids (B), triterpenes/steroids (C), flavonoids (D), and anthracenosides (E)

Species	A	B	C	D	E
<i>D. elliptica</i>	-	-	+	-	+
<i>L. paniculata</i>	-	-	+	-	-
<i>A. occidentale</i>	+	-	+	-	+
<i>R. ferruginea</i> (stem)	-	-	+	-	+
<i>R. ferruginea</i> (sprout)	-	-	+	-	+
<i>A. dasycarpum</i>	-	-	+	-	-

+ presence of the compound, and - absence of the compound.

Table 3 - Phytochemical prospecting of ethanolic extracts of the studied species for the following compounds: coumarins (A), alkaloids (B), triterpenes/steroids (C), flavonoids (D), anthracenosides (E), tannins (F), reducing compounds (G), and anthocyanins (H)

Species	A	B	C	D	E	F	G	H
<i>D. elliptica</i>	-	+	+	+	-	+	+	+
<i>L. paniculata</i>	-	-	+	+	-	+	+	+
<i>A. occidentale</i>	-	-	+	+	-	+	+	+
<i>R. ferruginea</i> (stem)	-	-	+	-	-	+	+	+
<i>R. ferruginea</i> (sprout)	-	-	+	-	-	+	+	+
<i>A. dasycarpum</i>	-	-	-	+	-	+	+	+

+ presence of the compound, and - absence of the compound.

reducing compounds due to the formation of a brown precipitate; anthracenosides due to the development of a red coloration; and anthocyanins due to the appearance of greenish-brown or blue coloration.

The hexanic extract from leaves of *D. elliptica* showed positive results for triterpenes/steroids and anthracenosides, while the ethanolic extract showed positive results for alkaloids, triterpenes/steroids, flavonoids, tannins, reducing compounds, and anthocyanins.

Recent studies conducted by Candido (2016) with *D. elliptica* showed, through bioassays, a potential herbicide and insecticide activity. In addition to these activities, *D. elliptica* has a gastroprotective, antimicrobial, antinociceptive, anti-inflammatory, antitumor, anti-hemorrhagic, and immunomodulatory activity proven by in vivo and in vitro experiments from different plant preparations (Lopes et al., 2007; Azevedo et al., 2007; Kushima et al., 2009; Nishijima et al., 2009; Carli et al., 2009).

Positive results were observed for triterpenes/steroids in the hexanic extract from the bark of *L. paniculata*, as well as positive for triterpenes/steroids, flavonoids, tannins, reducing compounds, and anthocyanins in the ethanolic extract.

Calixto Jr et al. (2016) also reported the presence of flavonoids in extracts of *L. paniculata*. Several activities have already been described on this species, such as antibacterial, antioxidant, and antifungal action (Moura-Costa et al., 2012; Lima Neto et al., 2015; Calixto Jr et al., 2015), but with no reports on its pesticide activity. However, the presence of flavonoids may be an indication of an allelopathic potential since phenolic acids are proved to have an allelopathic potential to inhibit the germination of seeds from different weeds (Santos, 2011).

The hexanic extract of leaves of *A. occidentale* was positive for coumarins, triterpenes/steroids, and anthracenosides, while the ethanolic extract was positive for triterpenes/steroids, flavonoids, tannins, reducing compounds, and anthocyanins.

The species *A. occidentale* is widely used in the therapeutic treatment of tooth pain, as an anti-inflammatory agent for gum and throat, arthritis, cramps, and bronchitis. The phytochemical

study showed it was the only species tested positive for coumarins, in addition to triterpenes/steroids and flavonoids. Coumarins and phenolic compounds have allelopathic action. A study carried out by Vyvyan (2002) on allelochemicals compounds showed that the main substances with allelopathic potential that act in both germination and plant development are benzoquinones, coumarins, lignoids, terpenoids, lactones, mucilages, tannins, alkaloids, and flavonoids. In addition, coumarin has a great therapeutic interest due to its antibiotic, bronchodilator, fungicide, anticoagulant, vasodilator, spasmolytic, and antithrombotic activities.

Chaves et al. (2010) also observed the presence of in natura steroids in ethanolic extracts of the integument of *A. occidentale*. This species has proven antimicrobial and antioxidant activity (Melo et al., 2006; Silva et al., 2007; Broinizi et al., 2007). Bioassays performed by Matias et al. (2017) with the bark of cashew nuts of the species *A. occidentale* proved its phytotoxic action on seed germination and root and seedling growth.

Extracts from stem and sprout of *R. ferruginea* did not differ in the assessed tests. Triterpenes/steroids and anthracenosides were observed in the hexane extract and triterpenes/steroids, tannins, reducing compounds, and anthocyanins were observed in the ethanolic extract.

Despite the presence of several classes of compounds in *R. ferruginea*, there are only studies on its antimalarial activity (Andrade Neto et al., 2003).

The hexanic extract from the bark of *A. dasycarpum* was positive for triterpenes/steroids and anthracenosides, and for flavonoids, tannins, reducing compounds, and anthocyanins in the ethanolic extract.

The species *A. dasycarpum* has been little studied and only its antibacterial activity is reported (Sousa Jr et al., 2009).

All the studied species had triterpenes/steroids, tannins, reducing compounds, and anthocyanins. Triterpenes are the most important group of the terpenoid class. Oliveira et al. (2014a) demonstrated the allelopathic effect and the negative interference in the development of root and shoot, and growth of lettuce seedlings using an ethanolic extract of the species *Pouteria ramiflora*, rich in triterpenes and steroids at different extract concentrations. Triterpenes are also recognized by anti-inflammatory, analgesic, cardiovascular, and antitumor effects (Simões et al., 2007).

Anthracenosides in plants are associated with flower pigmentation and, under a pharmacological aspect, are used therapeutically as laxatives and cathartics due to their action in irritating the large intestine, increasing intestinal mobility and, consequently, reducing water reabsorption.

Tannins have been the subject of a number of studies and most of them have been addressing ecological interactions between plants and herbivores since tannins may decrease the predation rate because it becomes unpalatable, moving away from natural predators (Paes et al., 2002; Monteiro et al., 2005). Researches on the biological activity of tannins have shown important action against certain microorganisms, as carcinogenic and liver toxicity causing agents (Chung et al., 1998). These latter effects undoubtedly depend on the dose and type of ingested tannin. The ingestion of green tea and diets rich in fruits that contain tannins, for instance, have been associated with the anticarcinogenic activity. In addition, tannins may act as anti-inflammatory and healing agents, and even as reverse transcriptase inhibitors in HIV (Kilkuskie et al., 1992).

The studied species presented several compounds that may be of great interest in the most diverse areas of studies of plant extracts, especially related to compounds with pesticide activity. Because these species are commonly found in pasture areas and may exhibit some allelopathic activity, it is evident the need to broaden the studies on them.

The phytochemical prospecting of these five species collected in the Cerrado region confirmed the presence of different secondary metabolites of interest, with the ethanolic extract being the richest in compounds with allelopathic potentials, especially *D. elliptica*, which presents seven classes of compounds, corroborating its widespread application in folk medicine and indicates its potential for use in other areas.

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