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Majoritary triterpenic compounds in some angiosperms of the central region of Rio Grande do Sul state

Fernanda Brum Pires [I]; Carolina Bolssoni Dolwitsch [II]
Marcella Emília Petra Schmidt [III]; Marina Zadra [IV]; Liliana Essi [V]
Camilo Amaro de Carvalho [VI]; Lucas Minoruk Frescura [VII]
Marcelo Barcellos da Rosa [VIII]

ABSTRACT

Five species of angiosperms collected in the Central Region of Rio Grande do Sul was evaluated due to their pharmacological properties. The objective of this study was to extract and characterize the triterpenic content of angiosperm species. The extracts were obtained by ultrasonic extraction using chloroform as solvent. Identification and quantification were performed using high performance liquid chromatography with UV detection (HPLC-UV). The presence of arjunic acid, maslinic acid, oleanolic acid, erythrodiol, uvaol, lupeol, β-amirin, α-amirin, stigmasterol, and β-sitosterol was observed. All species presented β-sitosterol. Polygala pulchella presented the greatest diversity of triterpenic compounds, while Ruellia angustiflora and Moquiniastrum mollissimum the least. Paspalum rawitscheri and Hesperozygis ringens presented the same constituents, differing from each other only quantitatively. Therefore, an overview of the triterpene constitution in species of the central region of the state of RS is presented, along with a review on the pharmacological properties of the investigated compounds. The results obtained in this work are relevant since they

[I] Federal University of Santa Maria, Santa Maria, Brazil. fernandabrumpirs@gmail.com
[II] Federal University of Santa Maria, Santa Maria, Brazil. caroldol@gmail.com
[III] Campinas State University, Campinas, Brazil. marcellaeps@gmail.com
[IV] Federal University of Santa Maria, Santa Maria, Brazil. marizadra@yahoo.com.br
[V] Federal University of Santa Maria, Santa Maria, Brazil. liliana.essi@ufsm.br
[VI] Federal University of Viçosa, Viçosa, Brazil. camiloamaro@yahoo.com.br
[VII] Federal University of Santa Maria, Santa Maria, Brazil. lmironuk15@gmail.com
[VIII] Federal University of Santa Maria, Santa Maria, Brazil. marcelo.b.rosa@ufsm.br

stablish an overview of the chemical constitution of the extracts whose biology is still little known. The pharmacological potential of the species can be attributed the identified triterpenic constituents thus supporting the use for medicinal purposes.

Keywords: Triterpenes; Moquiniastrum; Paspalum; Hesperozygis; Ruellia; Polygala

RESUMO

Cinco espécies de angiospermas coletadas na Região Central do Rio Grande do Sul foram avaliadas tendo em vista às suas propriedades farmacológicas. O objetivo deste estudo foi extrair e caracterizar o conteúdo triterpênico de espécies de angiospermas. Os extratos foram obtidos por extração ultrasônica usando clorofórmio como solvente. A identificação e quantificação foram realizadas por cromatografia líquida de alta eficiência com detecção UV (HPLC-UV). Foi verificado a presença de ácido arjunic, ácido maslínico, ácido oleanólico, eritrodiol, uvaol, lupeol, β-amirina, α-amirina, estigmasterol e β-sitosterol. Todas as espécies apresentaram β-sitosterol. Polygala pulchella apresentou a maior diversidade de compostos triterpênicos, enquanto Ruellia angustiflora e Moquiniastrum mollissimum o mínimo. Paspalum rawitscheri e Hesperozygis ringens apresentaram os mesmos constituintes, diferindo entre si apenas quantitativamente. Portanto, é apresentada uma visão geral da constituição dos triterpenos em espécies da região central do RS, juntamente com uma revisão das propriedades farmacológicas dos compostos investigados. Os resultados obtidos neste trabalho são relevantes, pois estabelecem uma visão geral da constituição química dos extratos cuja biologia ainda é pouco conhecida. O potencial farmacológico da espécie pode ser atribuído aos constituintes triterpênicos identificados, apoiando assim o uso para fins medicinais.

Palavras-chaves: Triterpenos; Moquiniastrum; Paspalum; Hesperozygis; Ruellia; Polygala

1 INTRODUCTION

Medicinal plants have been used for centuries in traditional medicine and are associated with promoting health and preventing/curing diseases (LASZCZYK, 2009; ROMERO et al., 2010).

In Brazil the use of medicinal plants is eased by the fact that the country presents the greatest biodiversity of the planet, resulting in a multitude of active principles (CHISTÉ et al., 2013). In contrast, it is known that many of the plants of this biodiversity are only little studied.

According to the Brazilian Institute of Geography and Statistics (IBGE, 2004), presents six distinct biomes. All with high biodiversity and phytophysiognomic diversity, but some of them are still poorly investigated and have been the focus of many studies in the last decade. One of these biomes is the Pampa, which covers only the state of Rio Grande do Sul in Brazil. In the last decades, about half of the area originally covered with fields in the state of Rio Grande do Sul has been transformed into other types of vegetation cover (PILLAR, 2009). This degradation is particularly worrying, since, despite recent advances, most of the Brazilian Campos Sulinos remains insufficiently known (GIULIETTI et al., 2005). Besides, out of the approximately 2,220 species of plants in the fields of Rio Grande do Sul, 213 are considered endangered (PILLAR, 2009), what makes more urgent the task of gathering knowledge and chemical characterization of this biodiversity.

The terpenes are natural hydrocarbons produced by a wide variety of plants and animals. They act in the defense and adaptation system of plants, show important pharmacological properties (Table 1), and therefore, are products of high interest for the chemical-pharmaceutical industry (RAMAWAT; MÉRILLIN, 2013). The triterpenes are terpenes which are composed by thirty

carbons (C_{30}) with promising pharmacological properties (MUFFLER et al., 2011). The triterpenic compounds involved in this study have their chemical structures represented in Figure 1 and their main pharmacological activities presented in Table 1.

Figure 1 - Chemical structures the compounds involved in this study

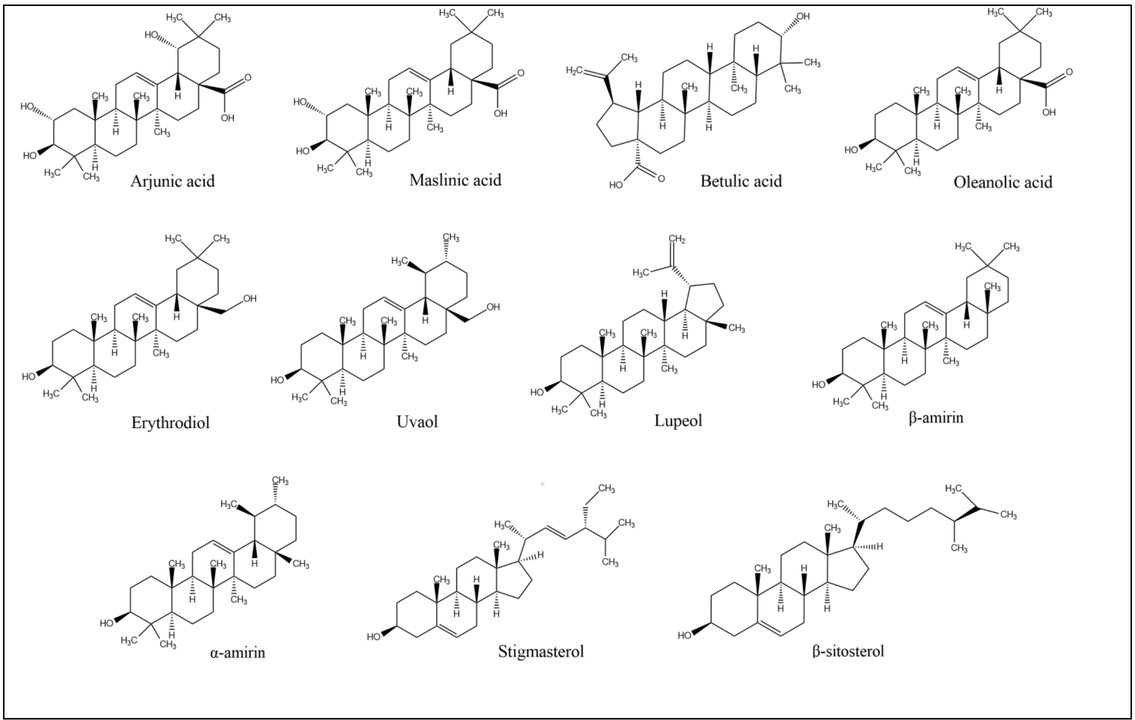


Table 1- Triterpenic compounds and their pharmacological activities:

Triterpenes	**Pharmacological Activities**
Arjunic acid	*Antioxidant, antimicrobial, antibacterial, neuroprotective, antidiabetic, scarring, inhibits the growth of insects, cardioprotective, antifungal, antioxidant, anti-inflammatory, cytotoxic and antitumoral (JOO, 2016).*
Maslinic acid	Inhibits the growth of insects (SÁNCHEZ-QUESADA et al., 2013), antifungal, antidiabetic, gastroprotector (SÁNCHEZ-ÁVILA et al., 2009), antitumoral (SIEWRT and CSUK, 2014).
Betulic acid	Antiviral, acts against the growth of parasites, antibacterial, anti-inflammatory, inhibits the growth of cancer cells (PAI et al., 2011, antimicrobial, antioxidant (MORE et al., 2012), hepatoprotective (DONFACK et al., 2010) and antifungal (GARCEZ et al., 2016).
Oleanolic acid	Anti-inflammatory, hepatoprotector, antitumoral, antimicrobial (MARTELANC; VOVK; SIMONOVSKA, 2009) antiviral, antifungal, antidiabetic, gastroprotector, reduces blood lipid levels (SÁNCHEZ-ÁVILA et al., 2009), antioxidant (DONFACK et al., 2010, antiprotozoal (XU; SU; ZANG, 2012) and antitumor (SHANMUGAM et al., 2014).
Erythrodiol	Antioxidant and antithrombotic (ALLOUCHE et al., 2010), antitumoral (SÁNCHEZ-QUESADA et al., 2013), antibacterial (DOUGLAS, 2016).
Uvaol	Antioxidant and antithrombotic (ALLOUCHE et al., 2010), antibacterial (DOUGLAS, 2016), anti-inflammatory and antioxidant (AGRA et al., 2016).
Lupeol	Antiprotozoa, anti-inflammatory, anticarcerogenic, cardioprotective, antimicrobial, hepatoprotector (UMARI; KAKKAR, 2012), antiarthritic, antimutagenic, antioxidant, antimalarial (SALEEM et al., 2008), antidiabetic, nephroprotector (SIDDIQUE; SALEEM, 2011) antioxidant (SANTIAGO; MAYOR, 2014).

β-amirin	Gastroprotector, antipruritic, hepatoprotector (MARTELANC; VOVK; SIMONOVSKA, 2007), anti-inflammatory and analgesic (DIAS; HAMERSKI; PINTO, 2011).
α-amirin	Gastroprotector, antipruritic, hepatoprotector, anti-inflammatory (MARTELANC; VOVK; SIMONOVSKA, 2007), antiarthritic (DIAS; HAMERSKI; PINTO, 2011), reduces sensitivity to pain stimuli (BACKHOUSE et al., 2008) and antimicrobial (MORE et al., 2012).
Stigmasterol	Antitumoral (LEE et al., 2012), cholesterol reducer and gastroprotector (QUÍLEZ et al., 2010), antifungal (MBAMBO; ODHAV; MOHANLALL, 2012) and larvicidal (GADE et al., 2017).
β-sitosterol	Gastroprotector (QUÍLEZ et al., 2010), analgesic (SAEIDNIA et al., 2014), anthelmintic, antimutagenic (SAEIDNIA et al,. 2014; VILLASEÑOR et al., 2002) , antimicrobial (SANTOS; MORENO, 2004) antitumoral (LEE et al., 2012, LOMENICK et al., 2015) and anti-inflammatory (SAEIDNIA et al., 2014; LOMENICK et al., 2015).

Different species of angiosperms were collected for the purpose of this study, considering the occurrence in the region and lack of scientific information about these compounds. The selected species belong to five different families, commonly found in grassland formations or edge of forests of the biomes Pampa and Atlantic Forest in the central region of Rio Grande do Sul. Three rare species, listed in the national list of endangered species (BRASIL, 2008) and poorly investigated biology were selected Moquiniastrum mollissimum (Malme) G. Sancho, Paspalum rawitscheri (Parodi) Chase ex G.H. Rua & Valls, and Hesperozygis ringens (Benth.) Epling). The other two species Ruellia angustiflora (Nees) Lindau ex Rambo and Polygala pulchella (A. St.-Hil. & Moq.), on the other hand, present broader distribution and belong to genera with citations in traditional medicine.

Moquiniastrum mollissimum (=Gochnatia molissima (Malme) Cabrera) is a species of the Pampa biome, which in Brazil is restricted to the state of Rio Grande do Sul, that belongs to the family Asteraceae (FLORA DO BRASIL, 2020).

The genus Moquiniastrum includes 21 species. In Brazil, 19 species are found distributed in the main phytogeographic domains that include Cerrado, Pampa and Atlantic Forest. Ten of these species occur in the South region (LOMENICK et al., 2015). Strapasson et al. (2014) assigns the following constituents to some species of the genus: sesquiterpenes, diterpenes, triterpenes, coumarins, flavonoids, caffeic acid derivatives, acetylene, and amino acid.

Paspalum rawitscheri (=Thrasyopsis juergensii (Hack.) Soderstr. & AG Burm.), known as “capim-dos-descampados”, is an endemic grass of southern Brazil. This species is in the list of endangered species of Brazilian flora due to the progressive reduction of the number of individuals in known populations (RUA; VALLS, 2012; MARCHIORI, et al., 2014). Little is known about this species biology, so genetic, chemical and ecological studies are encouraged.

The genus Hesperozygis (Lamiaceae) arouses scientific interest for medicinal use, being already recognized by its characteristic odor (LAWRENCE, 1992). Among the most important species we find Hesperozygis ringens. It is an aromatic plant, endemic to southern Brazil and listed as a threatened species (BRASIL, 2008). Popularly, the plant is used as an insecticide, which has already been scientifically proven. Such activity is attributed to the pulegone, main component of its essential oil, which is found in concentrations above 80%. It is also reported in the literature that pulegone confers nematicidal, larvicidal, acaricidal and bactericidal activity to the plant (VON POSER, 1996; SILVA et al., 2013; SILVA et al., 2014). Chemical constituents such as α-pinene, sabinene, β-pinene, limonene, linalool and caryophyllene were reported by Silva et al. (2014). They also show antioxidant, anesthetic (SILVA et al., 2013), allelopathic, antiparasitic and antifungical (VON POSER, 1996).

Ruellia angustiflora is an herbaceous forest-edge species. Plants from the genus Ruellia present secondary metabolites such as flavonoids, steroids, triterpenes, coumarins and alkaloids (SAMY et al., 2015; CHOTHANI et al., 2010; SALAH et al., 2002). These are responsible for cardiovascular, antihyperglycemic, antibacterial, antioxidant, antinociceptive, anti-inflammatory, and gastroprotective activities, among others that are usually associated to some species of Ruellia (SAMY et al., 2015). Ruellia angustiflora occurs in Brazil, Uruguay, Paraguay and Argentina. It is popularly known as “flor-de-fogo”, due to the red color of its flowers (ALICE, 1995; FUHRO; VARGAS; LAROCCA, 2005). Its leaves are referred to as scarring in traditional medicine (ALICE, 1995).

Polygala is the most diversified genus of the family Polygalaceae, with approximately 725 species (LUDTKE; CHIES; MIOTTO, 2008). Chemical research studies of the genus have revealed the occurrence of a variety of secondary metabolites, such as xanthones, saponins, flavonoids, and coumarins, as mentioned by Johann et al.

(2012). In addition to these compounds, the species of Polygala are also characterized by the presence of methyl salicylate found in its roots, which is used as pain killer, mainly for muscular pain (ROCHA et al., 2012). Martins-Nucci et al. (2016) reported the use of these species in the treatment of pain and inflammation. This species is used in popular medicine for the treatment of bronchitis, asthma, whooping cough, rheumatism as well as antiofidic (MARQUES; PEIXOTO, 2007).

Therefore, this article aims to identify and quantify triterpenic in Moquiniastrum mollissimum, Paspalum rawitscheri, Ruellia angustiflora, Polygala pulchella and Hesperozygis ringens collected in distinct locations of the central region of Rio Grande do Sul. Also, we aim to present a literature review of the compounds investigated.

2 MATERIALS AND METHODS

2.1 Chemicals

All the chemical standards used, α-amirin (98%), β-amirin (98,5%), β-sitosterol (85%), stigmasterol (95%), lupeol (90%), uvaol (95%), erythrodiol (97%), oleanolic acid (97%), betulic acid (97%), arjunic acid (88%) and maslinic acid (95%), were of analytical grade from Sigma-Aldrich (St. Louis, MO, USA). The solvents acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) were of HPLC grade from Tedia (Fairfield, OH, EUA). The Chloroform ($CHCl_3$) used was of analytical grade, from Merck (Darmstadt, Germany). Stock solutions of α-amirin, β-amirin, uvaol, erythrodiol, oleanolic acid, arjunic acid and maslinic acid 1000 mg/l, stigmasterol 481 mg/l, lupeol 365 mg/l, betulic acid 196 mg/l and β-sitosterol 873 mg/l were prepared in methanol. The working analytical solutions for the analytical curve were obtained by diluting the analytical solutions in acetonitrile with the following concentrations: 0.7; 6.5; 12.2; 18.0; 23.6; 29.3 and 35.0 mg/l. All solutions were stored at -20ºC until the moment of analysis.

2.2 Plant Material

The species were collected from different locations in the Central Region of the state of Rio Grande do Sul. Endangered species were collected without compromising their reproductive structures (leaves only). Table 2 shows the species involved in this study, as well as the part of the plant that was collected, month and year of collection and location.

Table 2 - Collection data of the studied species

Species	**Collected part**	**Voucher**
Hesperozygis ringens (Benth.) Epling	Leaf	Brazil, Rio Grande do Sul, São Pedro do Sul: Morro Itaquatiá. *L. Essi* 612. 23. Oct 2013. (SMDB)
Paspalum rawitscheri (Parodi) Chase ex G.H. Rua & Valls	Leaf	Brazil, Rio Grande do Sul, Santa Maria: Em afloramento areníti Morro Pedra do Lagarto. *L. Essi 799 et al.* 15 Oct 2014. (SMDB)
Moquiniastrum mollissimum (Malme) G. Sancho	Leaf	Brazil, Rio Grande do Sul, Santa Maria: Distrito de Santo Antão, Pedra do Lagarto. *L. Essi 904, J. Schaefer & H.F. Menezes*. 25 Mar 2015. (SMDB)
Ruellia angustiflora (Nees) Lindau ex Rambo	Leaf	Brazil, Rio Grande do Sul, Santa Maria: Distrito de Santo Antão, estrada entre a Pedra do Lagarto e a Capelo de Santo Antão. *L. Essi 760 & J. Freitas*. 14 Oct 2016. (SMDB)
Polygala pulchella A. St.- Hil. & Moq.	root and aerial part	Brazil, Rio Grande do Sul, Santa Maria: em campo úmido modificado, atrás da FATEC. L. Essi 745. 16 Jul 2016. (SMDB)

The parts of interest were dried in oven at 40 °C until constant mass. Afterwards, they were ground in a knife mill, packed in waterproof packages and protected from light until the moment of analysis.

2.3 Extraction Procedure

The extractions were conducted according to the methodology described by Pires et al (2017) with some modifications regarding the extraction solvent. Plant extracts were prepared via ultrasound-assisted extraction (Bandelin, Sonorex Super

RK 510 H). Glass tubes containing approximately 0.2 g of the samples received 10 mL of the extraction solvent (chloroform) and were placed in an ultrasonic bath for 4 h at room temperature. The resulting liquid from the extraction was filtered with filter paper, placed in glass beaker and then oven-dried at 40 °C for evaporation of the solvent. Afterwards, they were resuspended in acetonitrile (HPLC) and filtered by a 0.22 μm membrane (Sorbline Tecnologie). The initial concentrations were maintained. The extracted samples remained stored at -30 ºC until the analysis. Before injection into the chromatograph, all samples were diluted to 0.01 g/ml with acetonitrile (HPLC grade).

2.4 Quantitative Analysis of Triterpenic Compounds by HPLC-RP

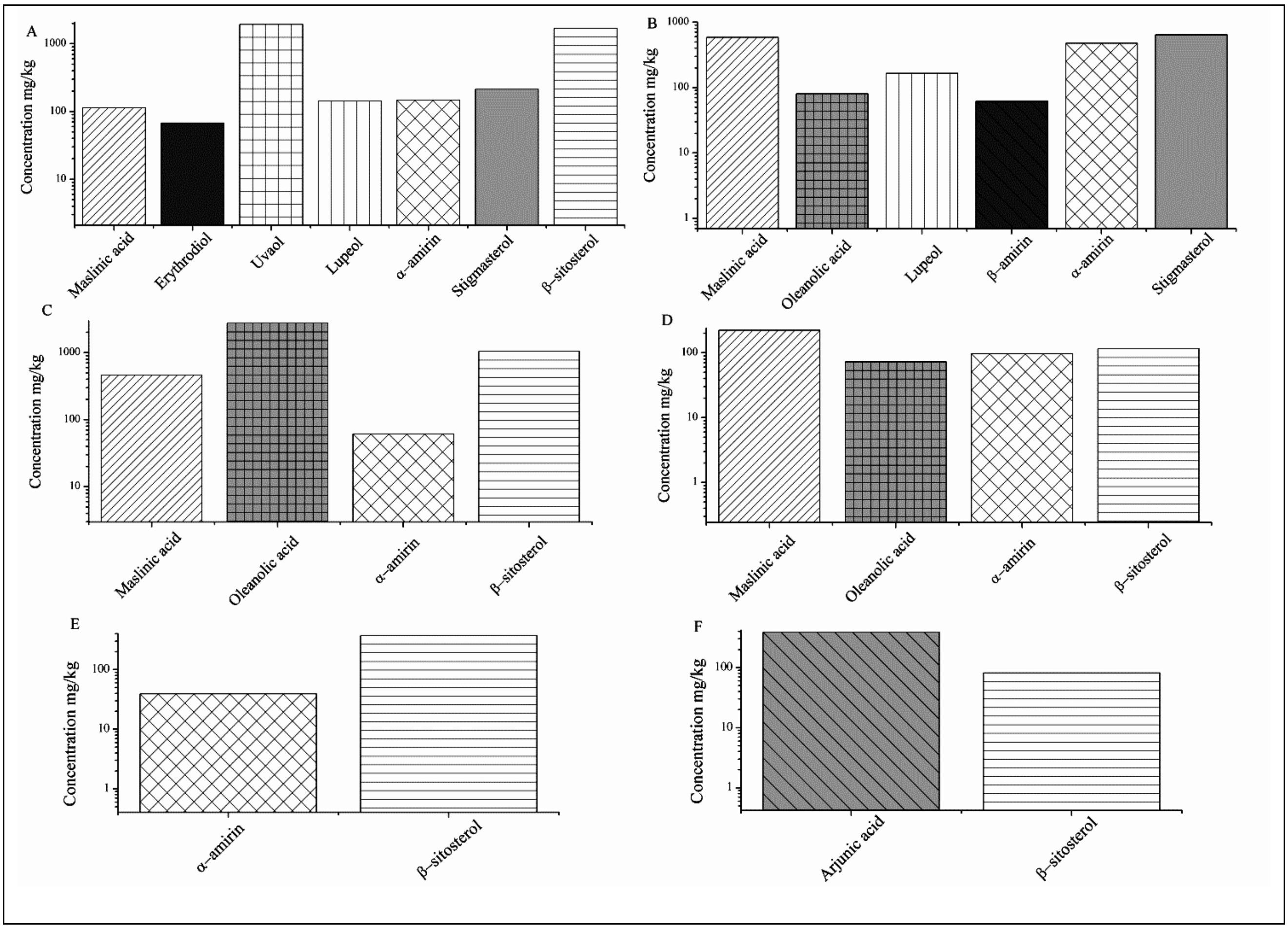
The identification and quantification of apolar constituents in the species were performed according to the method described by Schmidt et al (2018) that uses reverse phase chromatography and ultraviolet detection. Chromatographic measurements were performed on a Dionex® model P680 (Sunnyvale, CA, USA) liquid chromatograph equipped with a UV-vis detector model UVD170U, Rheodyne® injection valve model 8125 (Cotati, CA, USA) with loop of 100 μL. The analyses were carried out with a Kinetex reversed-phase C_{18} column (250 mm × 4.6 mm, I.D., 5 μm particle size; Phenomenex, Torrance, CA, USA) which was preceded by a Security Guard C_{18} pre-column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile: tetrahydrofuran (90:10, v/v) and the flow-rate was set at 0.5 ml/min. Spectrophotometry detection of the analytes was performed at the wavelength of 210 nm. Evaluation and quantification were made on a Chromeleon 6.7 Workstation. The general validation parameters were: linearity (0.74-40.0 mg/kg), LOD (0.06-1.04 mg/kg), LOQ (0.13-2.2 mg/kg), correlation coefficient r^2 (0.990-0.996) accuracy (80-120%), inter-day precision (1.84-4.40%) and intra-day precision (2.01-9.37%). All validation parameters are presented in detail in Schimdt et al (2018).

The quantification was performed using an external standardization method according to Ribani et al. (2004). The analytical curves were obtained by preparing the mixture of seven concentrations of the standards with injections in triplicates, where the analytical curve was expressed by the equation of the line. Samples were analyzed based on injections in triplicate, where the average of the areas corresponding to each compound presented by the sample was obtained. By taking the average of the area under the analytical curves, we obtained the concentration of the constituent in the sample.

3 RESULTS

The distribution of the triterpenic compounds in the investigated species are shown in Figure 2.

Figure 2 - A) Polygala pulchella (root), ***B)*** *Polygala* pulchella (aerial part), ***C)*** Hesperozygis ringens, ***D)*** Paspalum rawitcsheri, ***E)*** *Ruellia angustiflora,* ***and F)*** *Moquiniastrum mollissium*



4 DISCUSSION

Among the studied species, Polygala pulchella presented the greater diversity of compounds, as may be seen in graphs (a) and (b) in Figure 2. Although the literature mentions the roots much more often regarding medicinal purposes, it was verified that the aerial part is also rich in triterpenes (ROCHA et al., 2012).

The presence of β-sitosterol was verified in all species studied. This constituent was not found only in the aerial part of Polygala pulchella. β-sitosterol is described in the literature as presenting several pharmacological activities such as: hypocholesterolemic (CHAUHAN; RUBY; DWIVEDI, 2013), gastroprotective (QUÍLEZ et al., 2010), anti-inflammatory, analgesic (SAEIDNIA et al., 2014), anthelmintic, antimutagenic (VILLASEÑOR et al., 2002; SAEIDNIA et al., 2014) antimicrobial and antitumor (SANTOS and MORENO, 2004). The anthelmintic action of β-sitosterol may support the use of Hesperozygis ringens. Also, its anti-inflammatory and analgesic potential may endorse the use of Polygala pulchella.

In Polygala pulchella, in addition to β-sitosterol, β-amirin, α-amirin and lupeol may also justify the use of this species in the treatment of pain and inflammation (BACKHOUSE et al., 2008; DIAS; HAMERSKI; PINTO 2011; KUMARI; KAKKAR, 2012). Uvaol and erythrodiol were only identified in the roots of Polygala pulchella, and oleanolic acid only in its aerial part. Stigmasterol, maslinic acid, lupeol and α-amirin were found on both parts of the plant. Antifungal activity (SÁNCHEZ-ÁVILA., 2009) is attributed to the maslinic acid since it has already been proven in other species of the genus Polygala (JOHANN et al., 2012).

Polygala pulchella (aerial part), Hesperozygis ringens and Paspalum rawitscheri presented both oleanolic and maslinic acid. It should be highlighted that Hesperozygis ringens and Paspalum rawitscheri presented the same constituents, only differing quantitatively from each other, as shown in graphs (c) and (d) in Figure 2.

Moquiniastrum mollissimum and Ruellia angustiflora (Figure 2) presented lower diversity of compounds. However, the results are of great relevance due to the lack of studies about these species. *Moquiniastrum* mollissimum was the only species that presented arjunic acid.

Paspalum rawitscheri is also an unexplored species, but presented important constituents, as shown in the graphic (d) from Figure 2. This species needs further investigation, especially regarding its biology.

According to the results obtained, at least two triterpenic compounds are present in the chemical composition of the extracts of the analyzed species. Among the compounds investigated, β-sitosterol was the only one found in all species. Polygala pulchella presented the greatest variety of compounds. Hesperozygis ringens and Paspalum rawitscheri presented the same chemical composition, but in differed quantities. Moquiniastrum mollissimum and Ruellia angustiflora showed the least diversity of compounds. The results obtained in this work are relevant since they stablish an overview of the chemical constitution of the extracts. This allows the evaluation of the medicinal potential that such species may present regarding the presence of important triterpenic constituents with pharmacological activities. Our results also build knowledge around rare species, whose biology is still little known, drawing attention to the need for their conservation.

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