Foliar Phytochemistry of *Justicia Nodicaulis (Nees) Leonard (Acanthaceae)* occurrent in cerrado from Goias

Bioprospecção no cerrado: fitoquímica foliar de *Justicia Nodicaulis (Nees) Leonard (Acanthaceae)* ocorrente em cerrado goiano

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RESUMO

O gênero Justicia L. pode ser considerado o maior gênero de Acanthaceae, com cerca de 600 espécies. No gênero Justicia são relatados a presença de lignanas, flavanóides, terpenóides e alcaloides, que possuem grande importância na farmacologia. Neste estudo, o objetivo consistiu em observar e registrar as principais classes de metabólitos secundários das estruturas foliares da espécie Justicia nodicaulis (Nees) Leonard, ocorrente na área de Preservação Ambiental na Unidade Experimental do Centro Universitário de Anápolis – GO. Foram reunidas porções de folhas jovens (proporção de tamanho menor) e adultas (proporção de tamanho maior), sendo preferivelmente extraídas descensionalmente do terceiro nó, identificadas logo abaixo da extremidade da espécime, coletou-se amostras de 10 espécimes de J. nodicaulis (Nees) Leonard ao longo do córrego da área experimental do Centro Universitário de Anápolis. Materiais que foram adquiridos com o objetivo de realizar a prospecção fitoquímica e levantamento dos óleos essenciais. Para a análise qualitativa das classes de metabólicos secundários mais ocorrentes na espécie, utilizou-se metodologias descritas nas literaturas da ciência da fitoquímica. Entre os resultados, foram obtidos nas folhas novas e adultas, cumarinas, taninos, heterosídeos antraquinônicos, flavonóides, cardioativos e saponínicos. Entre o levantamento dos óleos essenciais foram obtidos 49 integrantes mais ocorrentes da literatura analisada.

Descritores: Cerrado; Justicia; Compostos Fitoquímicos; Botânica; Acanthaceae.

ABSTRACT

The genus Justicia L. can be considered the largest genus of Acanthaceae, with about 600 species. In the Justicia genus, the presence of lignans, flavonoids, terpenoids and alkaloids are reported, which are of great importance in pharmacology. The objective of this study was to observe and record the main classes of secondary metabolites of the leaf structures of the species Justicia nodicaulis (Nees) Leonard, occurring in the area of Environmental Preservation at the University Center of Anápolis - GO. Portions of young leaves (proportion of smaller size) and adults (larger size ratio) were collected, preferably being extracted descendingly from the third node, identified just below the end of the specimen, samples were collected from 10 specimens of J. nodicaulis (Nees) Leonard along the creek of the experimental area of the University Center of Anápolis. Materials that were purchased for the purpose of performing the phytochemical prospecting and survey of the essential oils. For the qualitative analysis of the classes of secondary metabolites most present in the species, we used methodologies described in the literature of the science of phytochemistry. Among the results, new and adult leaves, coumarins, tannins, anthraquinone heterosides, flavonoids, cardioactive and saponin were obtained. Between the survey of the essential oils, 49 members were obtained more occurring in the analyzed literature.

Descriptores: Cerrado; Justicia; Phytochemicals; Botany; Acanthaceae.

Introduction

Cerrado biome is home to more than 11,000 plant species, of which 4,400 are endemic, as well as a large variety of terrestrial and aquatic vertebrates and a high number of invertebrates. Phytochemical survey due to the medicinal importance among populations is constantly growing, equivalent to evolution scientific basis. Using medicinal plants has a very high socioeconomic relevance in the quality of life of low income communities due to their high availability, low toxicity, minimal risk of side effects and especially low costs and/or no burden compared to allopathic medicines.[1]

Genus Justicia L. can be considered the largest genus of Acanthaceae, with about 600 species, although it is not maintained as monophyletic, the lack of a generic revision that better establish the relations within the taxon has led to its use in sense in diverse studies, especially in regional floras.[2] According to Heywood (1985), some species of the Acanthaceae family are used for medicinal purposes.[3]

Studies referring to the medicinal potential for species of the genus Justicia refer basically to the species Justicia pectoralis Jacq., Popularly known as chambá or anador. Chambá shows an anxiolytic effect; related to the Gabaergic system; through depressor effect and has no collateral as a sedative[4]. Species of this study was Justicia nodicaulis (Ness). The aim of this research was to identify the secondary metabolites and to elucidate the chemical composition of the essential oils in the leaf extracts of J. nodicaulis (Ness) Leonard occurring in the Permanent Preservation Area of the Experimental Unit of the University Center of Anápolis, Anápolis, Goiás.

Method

Botanical material

Portions of young leaves (proportion of smaller size) and adults (proportion of larger size) were collected, being preferably extracted from the third node, identified just below the end of the specimen, samples were collected from 10 specimens of J. nodicaulis in each grouping along the stream of the experimental center of the University Center of Anápolis (16 ° 17'28.9 "S, 48 ° 56 ', 18.2" W). Leaves during collection in a plastic container presented abundant moisture when clustered, and while fresh they did not present necrosis, presence of fungi or chlorosis. Collections were always carried out in the specific region of the stream of the Experimental Area of the University Center of Anápolis after the bridge of the ecological trail of the toucan, always with the same group of the species J. nodicaulis, collected in the afternoon period.

Available exsicates under number 7,130 (UEG) are available at the LAPBIO (Research and Biodiversity Laboratory) of the University Center of

Anápolis and Herbarium of the State University of Goiás (UEG), Campus Anápolis.

Leaves were crushed in an organic electric shredder, Tog-Garthen Model 2300, until the appropriate particle size was determined for analysis.

Identification of secondary metabolites

To perform the phytochemical prospecting and evaluation of the essential oils, the leaves were air-dried for seven days and then microfragmented in the Tog-Garthen model 2300 organic grinder. Microfragmentation material obtained was stored in sterile transparent plastic bag, and later sealed, violating then, only at the beginning of the experiments. For the identification and description of the secondary metabolites, a methodology adapted from Matos[5], Matos & Matos[6] and Costa.[7]

Obtaining essential oil

A sample of 11.28 g of the microfragmented powder of the species Justicia nodicaulis was used. Essential oil of the species was extracted by hydrodistillation in modified Clevenger apparatus for 2 hours. Due to the small volume of oil obtained, 10 ml of 99.8% PA ACS 1000 ml of the brand ALPHATEC was used in order to obtain the best use of the extracted oil. Sample evaporated from the ether and sample weight was calculated by the difference between the flasks with and without the sample. It was then stored in a freezer for subsequent analysis.

Chemical composition of essential oil

Extracted essential oil can be analyzed by gas chromatography with mass spectrometry (CG/MS) and Retention Indices (IR) were calculated according to the literature values of the most common essential oils components[8]. A CG / MS (Shimadzu QP5050) equipped with capillary column (CBP-5; 30 cm x 0.25 mm x 0.25 mm), 1.0 ml.min-1 flow of Helium and programmed temperature (60 °C/2 min, 3°C min -1/ 240°C, 10° min -1, 280° C/ 10 min). The electron impact ionization of 70 eV with a mass range between 40-400 m/z due to sampling of 1.0 scan/s. The compounds were identified by computerized database, using digital mass spectral data library (National Institute of Standards and Technology, 1998), and by comparison with their retention indices and authentic mass spectra[9], relativos à coinjeção do óleo essencial com uma mistura de hidrocarbonetos, C8–C32, e com aplicação da equação de Van Den Dool e Kratz.[10]

Results and discussion

Identification of secondary metabolites

On the analysis developed, we can observe the process of anthraquinones, cardiac and saponin heterosides, flavonoids, tannins and coumarins. In the qualitative assay for the detection of anthraquinone heterosides, it was observed in tubes (I) acidified medium with HCl and (II) not submitted to acidification, the formation of a thin ring of dark reddish-brown coloration. Bornträger test is often used for the detection of free anthraquinones, where pink, red or violet staining is developed in basic media. After observing the colorations it was possible to conclude that the vegetal raw material of the analyzed sample had anthraquinone heterosides. Free anthraquinone compounds are soluble in organic solvents, so they pass into the ethereal phase of the test tubes, in addition to reacting with ammonium hydroxide, forming ammonia phenomena, a pink coloration that intensifies with the passage of time, becoming red. In the acid hydrolysis, the anthraquinones were released in combination, so that the presence of anthraquinone heterosides was detected in the tube (I) and in the tube (II) the presence of free anthraquinones was detected. In the study of cardioactive heterosides, all the characterization reactions were positive, some with greater intensity. It was observed that in the Liebermann-Burchard reaction the extractive solution was stained in brown, dark brown to greenish. In the Keller-Kiliani reaction, a reddish-brown ring formed in the contact zone and the acetic layer was colored greenish-brown. In Kedde's reaction, the extractive solution showed brown-blackish coloration to intense red. In the characterization reaction of the steroid nucleus, yellow fluorescence was observed under ultraviolet light 365nm. Due to the colorations observed in the described reactions, it could be suggested that the raw material of the J. nodicaulis species analyzed has cardioactive heterosides.

In the study of flavonoid heterosides all characterization reactions were positive. In the studied species, it was observed that in the reaction of Shinoda, the extractive solution of the samples presented red-brick color to reddish-brown. In the oxalo-boric reaction, yellow fluorescence was observed under ultraviolet light 365nm. In the reaction with concentrated sulfuric acid, yellow-lemon fluorescence was observed under ultraviolet light 365nm. In the reaction with aluminum chloride yellow-green fluorescence was formed under ultraviolet light, wavelength 365nm. In the reaction with hydroxides, extractive solution presented yellowish-green and yellowish-brown coloration. In the ferric chloride reaction, extractive solution of the samples showed green-brown to blackish-blue to blackish. This was probably due to the fact that the ferric chloride complexes with the hydroxyls present in the flavonoid derivatives, forming colored products. In the study of saponin heterosides, persistent foam formation occurred even after the addition of dilute mineral acid. Foaming occurred mostly from 6 ml of extractive solution. In the characterization of tannin the reaction with gelatin was positive, forming white precipitate. In the coumarin research, it was observed that in the region of the filter paper containing the ethereal phase and a drop of 1M NaOH, yellow-green fluorescence was produced under ultraviolet light of 365 nm wavelength.

Essential oil yield

In the analysis of yield of oil extracted on the leaves of J. nodicaulis was 0.05%. In studies on the chemical composition of essential oils, the yield found for the Justicia pectoralis species collected in Guadeloupe, India, yield ranged from 0.4 to 0.3%. 11 In this sense, yields found for the different species of Justice sp.

Chemical composition of essential oil

In the analysis of the chemical composition of the essential oil of the species under study, in the vegetative stage, 49 components were identified, and the 8 main ones occurring in the Cerrado of Goiás are listed in Table 1.

Table 1- Principal components and percent content of essential oils of leaves of J. nodicaulis occurring in Cerrado Goiano.

Chemical component of essential oil	**Percentage (%)**				
	1	2	3	4	5
Isopulegol	1,29	9,3	1,23	0	1,7
Beta-bourborene	0,71	0	3,96	0	3,3
(E) -caryophyllene	1,93	3,6	18,6	2,7	6,2
Geranyl 2-methylbutanoate	8,09	6	0	0	0
Epoxide II humulene	0,65	0	0	1,8	0
Alfa-cadinol	0,66	3,2	0	2	0
Selin-11-em-4-alpha-ol	8,41	0	5,47	6,9	4,4
Eudesma-4 (15), 7-diene-1-beta-ol	0	0	2,59	2,6	1,9

Caption: 1-5: Samples of J. nodicaulis (Nees) Leonard

Of the 08 main components, 12.5% are monoterpenes and monoterpenes alcohols are the smallest percentage of the total. Sesquiterpenes correspond to 82.50% of the total components of the essential oil, and the sesquiterpenes alcohols are in a higher percentage, 62.50% of the total, corroborating results found by Falcão & Menezes (2003)[12] that state that monoterpenes and sesquiterpenes are the main components of the essential oils of the Lamiales order, in which the family Acanthaceae. In addition to medicinal importance these compounds and many of their derivatives due to the pleasant floral odor are used in perfumery and cosmetic products, for example, perfumes, lotions, soaps, deodorants and as flavorings in foods such as non-alcoholic beverages, ice creams and sweets [13]. Silva Santos et al. (2006), in a review work, performed a survey of the main compounds of essential oils, used industrially and mention that monoterpenes such as citronellol, citronellal, geraniol and linalool are used for fragrances.[14]

It is stated that linalool has a wide application in several areas.[15] It has now been widely used as the starting compound for several important syntheses, such as linaline acetate [16]; Prates et al., (1998) and Belaiche et al., (1995) bactericide and fungicide.[17-18] In medicine it has been successfully applied as a sedative [19] and its anticonvulsive properties are being analyzed.[20]

In a review work on the pharmacological actions of Plectranthus barbatus Andr. (Lamiaceae) compounds, it is stated that the compounds (E) - cariophilene and caryophyllene oxide have a variety of biological activities, including antibacterial (against Gram-positive, Gram-negative and acid alcohol resistant), antiviral, anthelmintic, antiprotozoal, anticancer, chemotherapeutic, antifertility, hyperglycemic.[21]

Studies have shown that sesquiterpene α-muurolol has antimicrobial activity, mainly in pathogenic fungi and gram bacteria (+/-).[21] Several of the chemical components found in the essential oil of the species studied, such as espatulenol, α-muurolol and selin-11-em-4α-ol, were examined for their carcinogenic, antifertility, myotoxic, phytotoxic and insecticidal properties in ethnopharmacological review, pharmacological and chemical properties of some families as Lamiaceae belonging to the same order as the Acanthaceae.[12]

Recognizing existence of chemical races is important when one thinks about the collection of medicinal and aromatic plants, both for chemical and pharmacological studies in which one tries to evaluate and to improve the capacity of these plants to produce certain chemical constituent.[22]

Conclusion

This study allowed the morphological description and the survey of the chemical composition and yield of the essential oil of the structures collected from J. nodicaulis

Considering the results obtained, this work could be used in later studies as a contribution to the understanding of the chemistry and ecology of the species J. nodicaulis, thus integrating a collection of records of the genus Justicia and family Acanthaceae.

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