Phytochemical Analysis of *Turnera Diffusa* Willd

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Abstract

Turnera diffusa WILLD is a plant used in folk medicine as a natural stimulant, and the objective of this research is to perform the phytochemical analysis of the secondary metabolites of the damiana of occurrence in the Juazeiro region, northern Bahia - Brazil. A phytochemical screening was performed from three Crude Ethanol Extracts (BSE), EEB1: leaves and flowers; EEB2: stems and EEB3: root. A thin analytical layer chromatography was performed using specific developers to detect each chemical class. Alkaloids, coumarins, anthranic derivatives, phenolic compounds, mono, sequi and diterpenes, naphthoquinones, triterpenes and steroids, saponins, hydrolyzed tannins and xanthines were identified in the three extracts. Only in the BSE of leaves and flowers observed the presence of antroquinones and aglycones. The secondary metabolites identified in the study evidence the pharmacological potential of Turnera diffusa. Phytochemical screening concluded the presence of fourteen classes of secondary metabolites in damiana, indicating important medicinal potential with pharmacological effects reported in the literature.

Keywords: Chromatography. Damiana. Secondary metabolites. Sorting.

I. INTRODUCTION

Medicinal plants have been used since antiquity, through empirical knowledge of their healing properties and have always represented a therapeutic option of great value. Thus, phytochemical analyses are important to scientifically prove the medicinal species of popular interest, aiming to identify, quantify and evaluate the secondary metabolites present in them (BESSA et al., 2013).

Phytochemical tests seek to understand the functions of these substances for bioecological interactions and the identification of pharmacologically active molecules (ARAÚJO et al., 2015). Highlighting that environmental, seasonal and soil factors can influence the concentrations of metabolites present in the plant, altering plant metabolism. In this sense, since the 4th century BC there have been

reports of norms for the collection of medicinal plants (GOBBO-NETO; LOPES, 2007).

The coordination and alteration of plant metabolites occur through seasonal, daily factors; intraplant, interplant and intraspecific independent of a genetic control, the expression may undergo modification due to the interactions between biochemical, physiological, ecological and evolutionary processes (LINDROTH; HSIA; SCRIBER, 1987; HARTMANN, 1996). Secondary metabolites represent a conjunction between plants and the surrounding environment and are often affected by environmental changes (KUTCHAN, 2001).

The time at which a plant is collected is the most important factor, since the active constituents are not constant all year round, and the composition of secondary metabolites of a plant can vary appreciably during the day/night cycle (GOBBO-NETO; LOPES, 2007). The age and development of the plant, the different plant organs, which are also important and can influence the total amount of metabolites and the relative proportions of the components of the mixture (BOWERS, 1993; HENDRICKS et al., 1997).

The genus *Turnera* is known in science for the presence of important secondary metabolites (SZEWCZYK; ZIDORN, 2014), especially in traditional Mexican medicine damiana, used as an aphrodisiac, for liver diseases, depression, anxiety, neurosis and as expectorant, stimulant (ALCARAZ; DELGADO, SLENDER; REAL, 2004; BARBOSA et al., 2017).

The incentive and appreciation of the economic potential of the plant can be real and effective, in the latter of all drugs used in Western medicine only 25% come from plants, some used as a drug or as a derivative of a product of natural synthesis, resulting in a model of development of new drugs through the biodiversity of nature (DE REZENDE et al., 2016).

It is also used to improve the flavor of desserts, ice cream, sweets and beverages (GARZA-JUÁREZ et al., 2011), in addition, *T. diffusa* has antioxidant activity similar to quercetin (SALAZAR, 2008). A phytochemical investigation identified 35 compounds in *Turnera diffusa,* among these: flavonoids, terpenoids, saccharides, phenolics and cyanogenic derivatives (ZHAO et al., 2007) and 24 isolated structures to investigate the anti-aromatase activity of *Turnera diffusa* (ZHAO et al., 2008).

Thus, the objective of this research was to perform the phytochemical analysis of the secondary metabolites of Damiana occurring in Juazeiro Bahia, submedia region of São Francisco northeast of Brazil.

II. METHODOLOGICAL PROCEDURES

1. Collection and identification of plant material

This research was developed through registration in the National System of Management of Genetic Heritage and Associated Traditional Knowledge - SisGen, under the number of Registration no. A652F54. "Damiana" (*Turnera diffusa*) were collected at 8:00 a.m. on September 24, 2019 in the Department of Technology and Social Sciences (DTCS) of the State University of Bahia (UNEB), Campus III, Juazeiro (BA) located at geographic coordinates 9º25'10" of south latitude and 40º29'16" of west longitude and altitude of 367 m.

According to the Köeppen classification, the climate of the region is classified as Bswh, which corresponds to the semiarid region. The average minimum temperature ranges from 18.4 to 22.2°C and the maximum from 29.6 to 33.9°C. The driest period of the year and the highest heatstroke occurs from August

to November. The average annual rainfall is 529 mm, with the rainy season concentrated between November and April (TEIXEIRA, 2010).

The collection site of the plants was georeferenced with GPS and the plants photographed, then recorded in a field booklet information related to the vegetable, such as growth habit, height, color and flower odor; Fertile branches of "Damiana" were standardized with 40cm of stems, placed on planks and stored in greenhouses at 700 C for 72h. After drying, the plant material was placed in cardboard with a previously established size with the herbarium plug. To identify the plant species, the exsiccate was compared with others already identified in the Herbarium of State University of Bahia - UNEB, Juazeiro - BA.

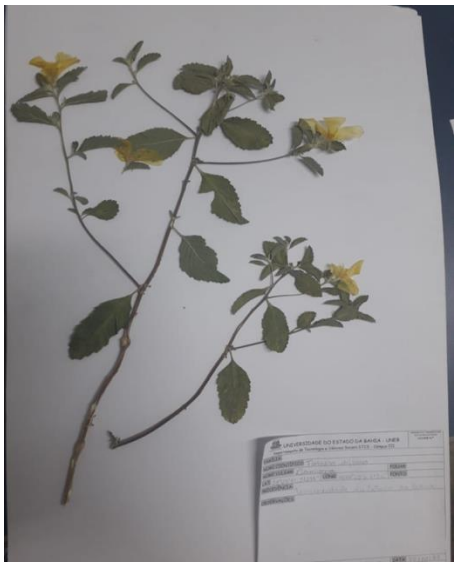

Figura 1. Exsicata da espécie *Turnera diffusa* WILLD depositada no Herbário da Universidade do Estado da Bahia. **Source:** Own Authorship.

The plant material was sanitized with running water and dried on paper towel by evaporation. The procedure of preparation and fractionation of crude ethanol extract (BSE) was developed in the laboratory of Pharmacognosy and Phytotherapy of UNIVASF.

The leaves and flowers, roots and stems of *Turnera diffusa* were dried in an oven at 40ºC for approximately 72 hours. Then, the leaves and flowers, stems and roots of the plant specimen were individually sprayed in a knife mill, generating three samples of plant drug, which were weighed and packed in exhaustive maceration systems with renewal of the extractor liquid (ethanol 99.5%) for seven days.

Crude ethanol extracts - BSE, being EEB1: leaves and flowers; EEB2: stems and EEB3: roots were obtained by evaporation of each extractive solution in a rotary evaporator at approximately 50ºC with reduced pressure (POSER; MENTZ, 2004).

EEB1, EEB2 and EEB3 were individually submitted to liquid vacuum chromatography using silica gel as a stationary phase and the solvents of increasing polarities hexane, chloroform, ethyl acetate and methanol as mobile phase, aiming at a pre-fractionation of the substances through their polarities.

2. Phytochemical screening of extracts

Based on the procedure of collection and selection of plant material, samples referring to different organs were investigated for phytochemical aspects. In this test the set of techniques used to track the compounds is based on staining reagents or precipitate formation that reveal the presence of secondary metabolites in an extract.

The analysis was performed in obtaining the crude ethanol extract (BSE) of *Turnera diffusa* by Analytical Thin Layer Chromatography (CCDA) with silica gel, in aluminum support (MachereyNagel®) using eluent and revealing system specific to each class of compounds.

For specific classes of chemical constituents, such as: alkaloids, coumarins, anthracene derivatives, phenolic compounds, mono, sequi and diterpenes, naphthoquinones, triterpenes and steroids, saponins, anthocyanins, lignans, antroquinones and aglycones, condensed tannins, hydrolyzed tannins and xanthines, tests were performed to identify the presence of these metabolites in the BSE according to wagner & bladt's adapted methodology (1996) (adapted).

III. RESULTS AND DISCUSSIONS

Phytochemical screening Through preliminary phytochemical screening by slender analytical layer chromatography (CCDA) was found the presence of classes of secondary metabolites in different organs of *Turnera diffusa* described in Table 1.

Table 1. Identification of the main classes of constituents of crude ethanol extract (BSE) in different organs of *Turnera diffusa* WILLD.

Chemical Class	Leaf and Flowers	Stalk	Root
Alkaloids	++	+	+
Coumarins (coumarin derivatives)	++	+	++
Anthracene derivatives	+	+	+
Phenolic compounds (flavonoids and phenylpropanoglycosides)	+	+	+
Mono, sequi and diterpenes	++	++	+
Naphthoquinones	++	+	+
Triterpenes and steroids	+++	+	+
Saponins	+	+	+
Anthocyanins	+	+	-
Lignans	+	+	++
Anthroquinones and aglyclones	+	-	-
Condensed tannins	-	+	+
Hydrolyzed tannins	++	+	+
Xanthines	+	+	++

(-); absence of the constituent, (+) presence of the constituent, (++); moderate presence of the constituent, (+++); high presence of the constituent. **Source:** Own Authorship.

The results show that the phytochemical extraction of "Damiana" was positive for the 14 classes of secondary metabolites tested. The presence of different chemical substances is linked to some medicinal uses, coupling several therapeutic purposes, it is also linked to the adaptation of the plant species to the environment (ROCKENBACH et al., 2018).

The crude ethanol extract (BSE) of leaves and flowers presented a higher incidence of secondary metabolites, highlighting a high level of concentration of triterpenes and steroids (Figure 2), which are part of a group of hormones and enzymes that interact for life maintenance. A similar study developed by Barbosa & colaboradores (2017) showed the presence of these constituents, highlighting the higher concentration of triterpenes and steroids in BSE of stalks and bark of medicinal plants.

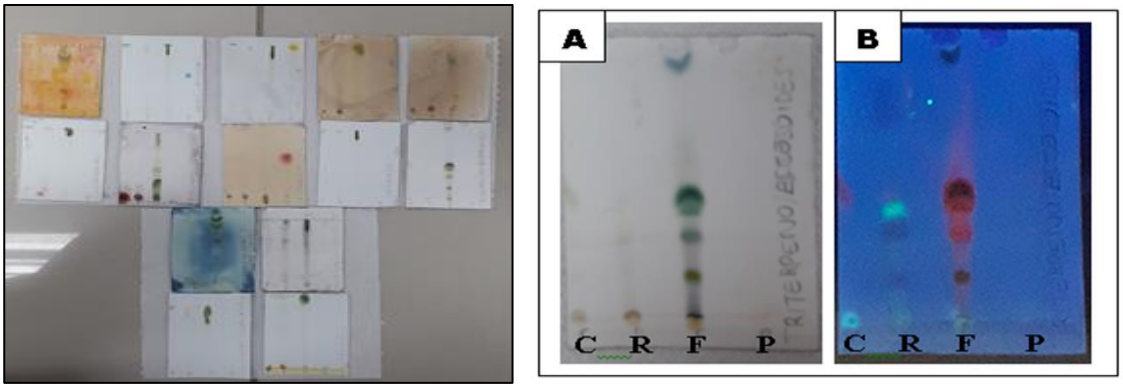

Figure 2: Phytochemical screening of *turnera diffusa* WILLD crude ethanol extract EEB. Identification of triterpenes and steroids (A) and UV 365 (B) of different plant organs (stem = C; root = R; leaf and flower = F and pattern = P). **Source:** Own Authorship.

The weak presence of anthracene derivatives, phenolic compounds and saponins in the three extracts evaluated was evidenced. And absence of condensed tannins in leaf and flower extract, antroquinones and aglycones in stem extract and absence of anthocyanins and antroquinones and aglycones in root extract. The other chemical classes evaluated had moderate presence in the evaluated extracts. Studies already reported in the literature show that arbutin and apigenin flavonoids are the main chemical constituents of *Turnera diffusa* with cytotoxic activity, present in methanolextract (AVELINO-FLORES et al., 2015).

Another study revealed that aceon extract also showed positive cytotoxic potential for MM cancer cells (WILLER et al., 2019). Apigenin-7-O-β-D-p-coumaroil flavonoids (1→6) glucopiranoside and p-arbutin have also been identified in 96% EtOH extract and infusion (CAMARGO; VILEGAS, 2010).

Study developed by Szewczyk & Zidorn (2014), composing the presence of 22 flavonoids, maltol glycolide, 7 cyanogenic glycosides, monoterpenoids, sesquiterpenoids, triterpenoids, polyterpene, fatty acids and caffeine in the genus Turnera. Flavonoids are capable of inhibiting cytochrome P 450 enzymes, which interferes with antiretroviral therapy (PIACENTE et al., 2002; Lee, Lee; ANDRADE, MR. ANDRADE; FLEXNER, 2006).

According to Zhao et al., (2007) "Damiana" contains many flavonoids, some with relatively high concentrations. The importance of these compounds consists of several beneficial biological functions, such as antioxidant, anti-inflammatory and anticancer effects (MIDDLETON et al., 2000; NARAYANAN, 2006), most of these flavonoids inhibit the aromatase enzyme, with flavonon pinocembrina with the

strongest inhibitory activity (ZHAO et al., 2008).

The hydroethanolic extract of T. diffusa identified flavonoids, specifically a mixture of flavones with aromatic acids and flavonols 3-O-diglucosides with high concentrations, in addition, presented cytotoxic activity in astrocytes cells (BEZERRA et al., 2016). However, the data obtained in the present study do not quantify the substances, it only proves the presence of chemical constituents in the BSE of different parts of the plant.

Studies confirm that plants containing coumarins (RESCHKE et al., 2007), tannins (MENDES et al., 2011), flavonols and flavanonas (DE PINHO et al., 2012), steroids and alkaloids (BESSA et al., 2013), in addition to phenolic compounds (FIRMO et al. 2014), can confer antimicrobial activity to an extract, thus *Turnera diffusa* presents these metabolites in their composition corroborating popular use and literature data.

Some secondary metabolites increase their concentration due to water stress, such as cyanogenic glycosides, glucosinolates, some terpenoids, alkaloids and anthocyanins (GOBBO-NETO; LOPES, 2007). In this sense, anthocyanins were found only in the BSE of leaves and flowers and in stem, absent at the root, which explains the positive correlation with the intensity of solar radiation and the presence of this class (JEONG et al., 2004).

Factors such as seasonality interfere practically in all classes of secondary metabolites since, in medicinal plants this concentration can change up to 80% (GOBBO-NETO; LOPES, 2007). The biosynthesis of secondary metabolites in medicinal plants has a direct relationship with genetic, environmental and agronomic factors. *Turnera diffusa* has pharmacotherapeutic potential and its metabolites may represent the opportunity for further research and development of effective and low-cost treatments from its molecules (SOUZA et al., 2017).

IV. CONCLUSIONS

The presence of secondary metabolites in *Turnera diffusa* WILLD identified from phytochemical analysis proves its pharmacological potential and reinforces traditional knowledge about its uses.

Turnera diffusa present in the sub-middle region of São Francisco has chemical properties relevant to the development of other research, both pharmacology and agronomic aspects.

This study suggests the isolation of these phytochemicals and in vitro tests for the safe use of the plant and its herbal medicines, as well as the study of seasonality and its primary metabolites.

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