Phenolic constituents of young plants of *Bauhinia forficata* Link

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RESUMO: Folhas de pata de vaca, *Bauhinia forficata* Link (Fabaceae) são usadas na medicina popular, no tratamento de diabetes . Considerando que atividade hipoglicemiante tem sido atribuída a alguns flavonóides e outros compostos fenólicos , o objetivo deste trabalho foi detectar a presença dos mesmos nos primeiros estádios de desenvolvimento de plantas de *B. forficata*, visando otimizar o uso desta espécie. Folhas de plantas de seis meses, cultivadas a partir de sementes, foram secas e pulverizadas para a extração de taninos e de flavonóides. A dosagem de taninos foi feita pelo método de difusão radial e a análise de flavonóides envolveu métodos cromatográficos convencionais e espectrometria de UV. Não foi detectada a presença de taninos e os principais flavonóides detectados foram glicosídeos de canferol.

Palavras-Chave: plantas medicinais, taninos, hipoglicêmicos

ABSTRACT: Leaves of *Bauhinia forficata* Link (Fabaceae) are used in the folk medicine for the treatment of diabetes. Considering that the hypoglicemiant activity has been attributed to flavonoids and phenolic compounds, the aim of this work was to detect the presence of them in the first stages of development of this plant , which could reduce the time of collecting and optimize its production. Leaves of six year old plants, grown from seeds, were dried and powdered for extraction of tannins and flavonoids. Tannins were quantified by radial diffusion assay and flavonoids were analysed by conventional chromatography methods and UV spectrometry. Tannins were not detected and the main flavonoids detected were kaempferol glycosides.

Key words- plants medicinal, Tannins, hypoglycemic agents

INTRODUCTION

Bauhinia is a cosmopolitan genus, comprising about 570 species, with equatorial, tropical, and subtropical distribution. The genus is represented in Brazil by 64 species occurring mainly in the rain forest and savannah ecosystems (cerrado) (Robertson & Lee, 1976). The study of this genus is relevant due to its taxonomic, phylogenic, ecological, and economic importance.

Among the species, *Bauhinia forficata* Link is genuinely Brazilian, represented mostly in Southeastern Brazil (Costa, 1942). In South America, particularly in Brazil and Argentina, the use in folk medicine of leaves of several *Bauhinia* species as antidiabetic is widespread. According to Marles & Farnsworth (1995), seven other species of the genus are cited to present hypoglycaemic activity. Juliani (1931) observed that leaf infusion of *B. forficata* progressively lowered glucose level in the blood of his patients and improved their general clinical state.

Among the chemical constituents found in members of the genus, the phytohemmaglutinins have been the most studied (Toms & Western, 1971). The genus has not been intensively investigated for low molecular weight organic compounds. Phenolic compounds and terpenic substances such as catequins, flavones and flavonols, campesterol, stigmasterol, and sitosterol, among others, were found in the genus (Gupta et al., 1980; Laux et al., 1985; Iribaren & Pomílio, 1987; Achenbach et al., 1988; Kumar et al., 1990).

The presence of tannins has been reported in many species of *Bauhinia*. Salatino (1977) found pirocatequinic tannins in the bark of *B. tonningii* and in leaves of *B. holophylla*. Although the therapeutic action of tannins has not been frequently reported, some are active against *Escherichia coli* and *Candida albicans* (which can cause diarrhea) and other microrganisms (Burapadaja & Bunchoo, 1995). Costa (1975) considers the possibility that the hypoglicaemic activity of some plants is due to the presence of tannins.

Abd-El-Wahab (1987) isolated quercetin, rutin, quercitrin, apigenin-7-*O*-glucoside from *B. purpurea*. The hydro-alcoholic extract from leaves of this plant reduced in 33.8% the glucose level in blood of diabetic rats. This activity was attributed to the flavonoids present in the extract.

Most of the investigation carried out with *Bauhinia*, used mature plant organs collected from

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their natural environment. It is worth considering, however, that secondary metabolites that accumulate in plants vary quantitatively and qualitatively, depending on the genotype, developmental phases, and also environmental factors. Concerning the importance of *B. forficata* and its compounds in anti-diabetic activity, the aim of this work was to investigate the presence of some phenolic compounds in the initial stage of development of *B. forficata*.

MATERIAL AND METHODS

Plant material

Young plants (six months old) of *B. forficata* grown from seeds, collected in Lavras (MG, Brazil), in April 1995, were used as experimental material The leaves were oven dried (50 °C) and powdered for extraction of tannins and flavonoids. A voucher specimen of the adult plant was deposited in the herbarium of UFLA (ESAL 07976).

Extraction and analysis of tannins

Extraction of powdered leaves (500 mg) in MeOH: H_2O (1:1) was conducted under refluxe for three times. The quantification of tannins was carried out according to Hargerman (1987). 15 µL of the extracts were deposited in cavities in an agarose gel (type 1 Sigma) containing 0.1%(w/v), bovine serum albumin (BSA). Tannic acid solution was used as standard in different concentrations. This method is based on the principle that the amount of precipitated tannin is proportional to the halo area formed around the cavities.

Extraction and isolation of flavonoids

These analysis were carried out according Mabry *et al* (1970) and Markham (1982). Extraction of powdered leaves was performed three times with refluxed 80% aqueous methanol for 60 min. The pooled extracts were concentrated under reduced pressure. The flavonoids were isolated through a poly-vinyl-polypyrrolidone column using Egger's solvent ($CHCl_3$: MeOH: MEK: Acetona; 20:10:5:1, v;v;v;v) and 80% aqueous MeOH, paper chromatography using OHAc 15% and BAW (*n*-BuOH: OHAc: H_2O; 6:1:2) as solvent system, and a Sephadex LH 20 column using MeOH.

Structural determination

The identification of the flavonoids involved the following steps:

a) Hydrolysis of the glycosides; b) Separation of the aglycones and sugars; c) Ultraviolet spectra of the glycosides and aglycones; d) TLC on cellulose plate of the aglycones and sugars; e) Chromatography and spectrometric comparison with standard compounds.

Extraction and detection of alkaloids

Extraction of powdered leaves was performed with 10% acetic acid in ethanol. After concentration of the extract to one-quarter of original volume NH4OH was added. The precipitate was screened for alkaloids by precipitation tests with Draggendorf's, and Mayer' reagents (Costa,1972; Harborne, 1984).

RESULTS AND DISCUSSION

Tannins

Although tannins have been reported in several *Bauhinia* species, the leaf extract of young plants of *B. forficata* did not present this class of compounds. This finding could be related to the developmental phase and the organ analysed, since tannins acumulate mainly in mature plants and specially in the bark.

Flavonoids

The analysis indicated the presence of one tri-glycoside (**1**) and one di-glycoside (**2**) of flavonol. Rf (x 100) in BAW and HOAc 15% of the first compound were 40 and 78, respectively, and 60 and 60 for the latter. After hydrolisis, the aglycones detected for both compounds presented the following UV spectral data in MeOH: 266, 299, 322, 365 nm, and in MeOH/KOH: 278, 322, 417 nm and by co-chromatography on TLC with standard compounds, the aglycone was shown to be kaempferol. The sugars were identified as glucose, rhamnose and xylose for (**1**) and glucose and rhamnose for (**2**) (Figure 1).

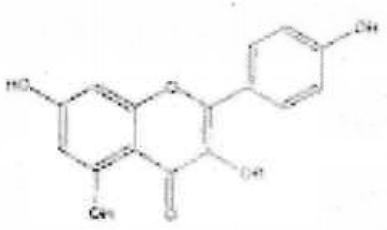

FIGURE 1 – Leaf flavonoids of six month old *B. forficata.* (**1**) kaempferol-3-*O*-(gly-xyl-rham)*. (**2**) Kaempferol-3-*O*-(gly-rham)*.
*the sequence of sugars was not determinated

UV spectral data of (**1**) in MeOH are: 266, 297, 348 nm and in MeOH/KOH: 274, 324, 396 nm; and of (**2**) in MeOH: 265, 297, 349 nm and in MeOH/KOH: 273, 325, 395 nm. Comparison of the spectral data in MeOH of the aglycones and glycosides showed a hypsochromic shift of band I of -17nm, indicating the link of sugars at C_3 hydroxil (Mabry *et al.* 1970). The above data led us to suggest the structure of (**1**) as kaempferol-3-*O*-(glucose-rhamnose-xylose) and (**2**) as kaempferol-3-*O*-(glucose-rhamnose).

Although modern analytical and spectroscopical techniques for the characterisation of natural products are now available, according to Bruneton (1995), the classical chromatographic and UV spectrometric methods provide reliable indication on the structure type, the nature and location of substituents.

Marles & Farnswhort (1995) reported the presence of daucosterol, lupeol and pectin for this species and attributed its hypoglycaemic activity to these substances. They also associated the presence of the different alkaloids to the hypoglycaemic activity by the plant. Our screening for alkaloid detection in young plants of *B. forficata* did not show the presence of this class of compound.

Salatino *et al.*, (1999), working with leaf extract in mature plants of *B. forficata* detected triglucosides of kaempferol, di and tri-glucosides of quercetin. According to our results, leaf extracts of young plants of *B. forficata* showed the presence of di and tri-glucosides of kaempferol. This results indicate that these flavonoids can be present during all the developmental cycle of this species.

Khosa et al. (1983) isolated from the bark extract of *Ziziphus rugosa* a fraction rich in kaempferol–3-*O*-rhamnoside and quercetin-3-*O*-rhaminoside which presented hypoglycaemic activity in rabbits. This way, the presence of kaempferol glycosides in leaves of *B. forficata* may be related to its efficiency against diabetes, a metabolic disease suffered by about 6% of world population and of which the mortality index due to this sickness keeps increasing (De la Fuente, 1995).

In this paper, we therefore suggest that the utilisation of leaves can be performed already in the first stages of plant development, reducing cultivation time and increasing the economic advantage for high scale production.

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