

## Botanical (morphological, micrographic), chemical and pharmacological characteristics of *Pfaffia* species (Amaranthaceae) native to South Brazil

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*Some parameters for the quality control of P. glomerata and P. paniculata roots using their botanical and chemical characteristics are presented. It was also carried out an in vitro pharmacological screening to evaluate some biological properties of P. glomerata that could be related to its popular use as "tonic". Relating to biological assays, ethanolic extract from P. glomerata roots did not present antiviral, antiproliferative, antifungal or MAO inhibitory activities. The cytotoxicity evaluation of P. glomerata determined that IC<sub>50</sub> is >2,000 µg/mL. The main morphological and micrographic characteristics of P. glomerata and P. paniculata roots are described in this paper in order to aid in their unequivocal identification.*

#### UNITERMS:

- *Pfaffia glomerata*
- *Pfaffia paniculata*
- Brazilian ginseng
- Micrography
- Quality control

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## INTRODUCTION

Around ninety species of *Pfaffia* (Amaranthaceae) are known in Central and South America, twenty-seven species have been described in Brazil (Vasconcelos, 1982; Taniguchi *et al.*, 1997). They are popularly known as "corango", "suma", "paratudo" and "Brazilian ginseng" since *Pfaffia* species are commonly used for the same therapeutic indications as ginseng (*Panax* spp. - Araliaceae) (Oliveira, 1986). Although *P. paniculata* is the most employed in commercial preparations in Brazil, *P. glomerata* is commonly used in the State of Rio Grande do Sul (South of Brazil) labeled either as *P. glomerata* or

wrongly as *P. paniculata*. A study of the differences between these two latter species is clearly needed in order to avoid adulterations that could result in undesirable or toxic effects.

Regarding chemical constituents from roots of *Pfaffia* species, allantoin, ecdysteroids, pfaffic acid and their glycosides (nortriterpene saponins), stigmaterol and sitosterol (Nakai *et al.*, 1984; Nishimoto *et al.*, 1984, 1988; Shiobara *et al.*, 1993 a,b; Takemoto *et al.*, 1983) have been identified.

Earlier pharmacological studies showed that ethanolic extract of *P. glomerata* roots possessed central nervous depressant activity (De Paris *et al.*, 1998b, 2000)

while crude extracts from *P. paniculata* displayed anti-inflammatory, sexual stimulant activities and low toxicity in rodents (Mazzanti, Braghirolli, 1994; Arletti *et al.*, 1999). In turn, triterpene saponins from *P. paniculata* showed to possess antitumoural activity (Nishimoto *et al.*, 1984).

In particular, one of the aims of this work is to provide botanical parameters for the quality control of *P. glomerata* and *P. paniculata*. In part due to difficult botanical differentiation among *Pfaffia* species, it is quite common the misidentification of the raw material in commercial preparations (Santos *et al.*, 1987; De-Paris *et al.*, 1998a). The major morphological features and the micrographic characters of *P. glomerata* and *P. paniculata* roots are presented here in order to give the necessary background for their unequivocal identification.

In addition, we discuss the thin-layer chromatography (TLC) profile of *P. glomerata* and *P. paniculata* species, and the antifungal, antiproliferative, antiviral, MAO inhibitory activities and cytotoxic properties of *P. glomerata* roots. These data could be relevant for future assessment of efficacy and safe use of *P. glomerata* in the traditional medicine of South Brazil.

## MATERIALS AND METHODS

### Plant Material

*Pfaffia glomerata* (Spreng.) Pedersen and *Pfaffia paniculata* (Mart.) O. Kuntze fresh roots were obtained from the cultivated area of the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), UNICAMP, Campinas, São Paulo, Brazil. For botanical characterization, the roots were collected on October 1999 by one of the authors (S.J.G.) and voucher specimens (*P. glomerata* Gattuso 754, *P. paniculata* Gattuso 755) are kept in both, U.N.R. Herbarium and in the Herbarium of the Vegetal Biological Area, of National University of Rosario, Argentina. For chemical and biological analysis, the roots were collected and identified at CPQBA on January 2000 by Ílio Montanari (*P. glomerata*, CPQBA 0238, *P. paniculata*, CPQBA 0241).

### Extraction Procedures

Roots and rhizomes (subterraneous parts) from *P. glomerata* and *P. paniculata* were reduced to small pieces, dried in circulating air stove (45 °C) and triturated. The powder was extracted with 60% ethanol under reflux (1:10, plant:solvent, w/v) during 6 hours. The ethanol was removed under "vacuum" and the resulting residue was lyophilized and stored under light protection. The yield of

both lyophilized extracts was 28% in relation to the dried subterraneous parts.

### Micrographic Studies

Fresh and fixed (formaldehyde:ethanol:acetic acid:water, 2:10:1:3.5) roots from both species were used. Longitudinal and transverse sections of the roots measuring 10 µm were prepared with microtome and stained with Safranin and Fast Green (Dizeo de Strittmater, 1979). Sections were mounted in synthetic balsam. Roots was macerated by conventional methods (Boodle, 1916). The distributions of calcium oxalate crystals was visualized by a polarizing microscope. Original drawings were made with a drawing device. Symbols proposed by Metcalfe and Chalk (1957) were used in the diagrams. The black and white photomicrographs were performed with the Axiolab Zeiss Automatic System.

### TLC Analyses

TLC was carried out on silica gel (Merck, GF<sub>254</sub>) using either chloroform:ethanol (95:5, v/v) or butanol:ethyl acetate:water:acetic acid (4:1:5:1, v/v). Compounds were visualized by heating (120 °C) using the anisaldehyde-sulfuric acid-sprayed plates. Ecdysterone, oleanolic acid, sitosterol and stigmasterol were used as authentic samples.

### Antiviral Activity of *P. glomerata*

Ethanollic extract was evaluated for cytotoxicity, antiviral and virucidal action according to Simões *et al.* (1999), using VERO cells (ATCC:CCL81) and *Herpes simplex* virus type 1 (HSV-1)/strains VR733 (ATCC), KOS and 29R/acyclovir resistant (Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de Rennes I, Rennes, France). To assess the effects of the extract, dilutions ranging from 2,000 to 1.9 mg/mL were prepared in 199 media.

### Antiproliferative Activity of *P. glomerata*

This test was performed following the methodology described elsewhere (Likhitwitayawuid *et al.*, 1993). The ethanollic extract was dissolved (serial dilutions ranging from 5 to 1000 µg/mL) in DMSO and added to the cell culture medium to give a final concentration of co-solvent of 0.1% (v/v). The following cell culture systems were used: A-375 human malignant melanoma, A-549 human non-small cell lung carcinoma, WiDr human colon carci-

noma, from the American Type Culture Collection (Rockville; MD, USA).

### Antifungal Assays of *P. glomerata*

The fungistatic activity was evaluated by the agar dilution method using Sabouraud-chloramphenicol agar for yeast, filamentous fungi and dermatophytes, according to reported procedures (Mitscher *et al.*, 1972; Zacchino *et al.*, 1998, 1999; Feresin *et al.*, 2001). The following strains were used: *Candida albicans* ATCC 10231, *Candida tropicalis* C 131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATCC 26934 and *Aspergillus niger* ATCC 9029. Dermatophytes: *Microsporum canis* C 112, *Trichophyton rubrum* C 110, *T. mentagrophytes* ATCC 9972, *Epidermophyton floccosum* C 114 and *Microsporum gypseum* C 115 kindly provided by CEREMIC, Centro de Referencia Micológica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531, Rosario, Argentina. The extract was solubilized in DMSO and added to the culture medium to give a final concentration that does not exceed 2% (v/v) to give serial decreasing dilutions ranging from 1000 to 250 µg/mL.

### MAO Inhibitory Activity of *P. glomerata*

The ethanolic extract was assayed for monoamine oxidase A (MAO A) and MAO B inhibitory activity in rat brain mitochondrial preparations at concentrations ranging from 1 to 20 mg/mL, following the methodology described by Gnerre *et al.* (2001). The extract was dissolved in DMSO and added to the cell culture medium to give a final concentration of co-solvent of 5% (v/v).

## RESULTS AND DISCUSSION

### Morphological and micrographic analyses of roots from *Pfaffia glomerata* (Spreng) Pedersen and *Pfaffia paniculata* (Mart.) O. Kuntze

Morphological and micrographic descriptions of the main characteristics of these plants are necessary to provide unique insights into unequivocal recognition of *P. glomerata* and *P. paniculata*.

#### Common characteristics of the roots

Both species consist of underground swollen roots, the outer surface is yellow-brown to brown and transversely rugose. The transverse root sections show a narrow

bark composed of almost tabulated phellem cells. The anatomical peculiarity is the anomalous growth in thickness, which takes place by the development of a succession of collateral vascular bundles from rings of secondary meristematic tissue. The secondary bundles are embedded in conjunctive parenchyma. Subsequently, the accessory cambium arises from parenchyma cells on the periphery of the phloem of each vascular bundle. The secondary phloem was composed of sieve tube elements, companion cells, and axial parenchyma. The secondary xylem was composed of vessel, tracheids, xylem fibres and axial parenchyma (Figure 1).

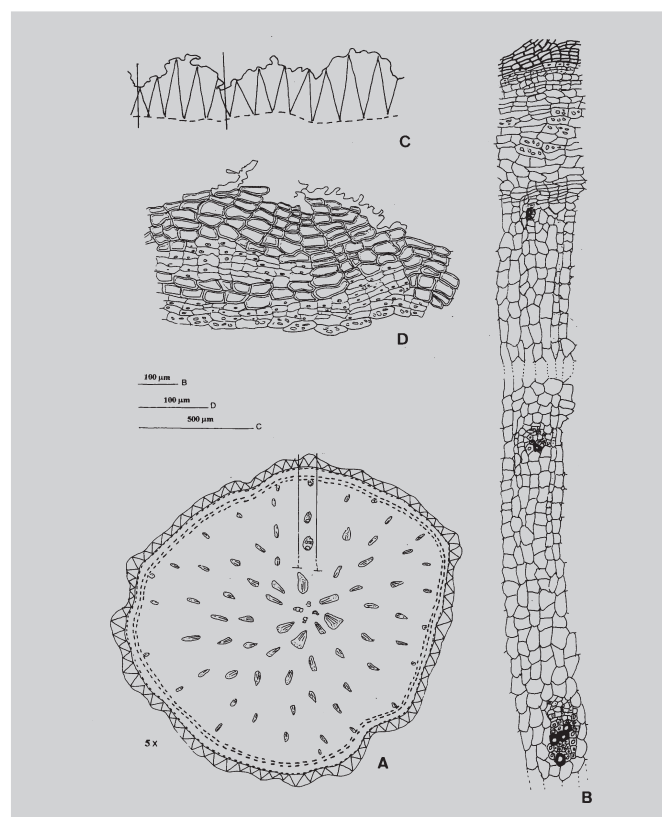


FIGURE 1 - A-D Common characteristics of *Pfaffia* Mart. A: Metcalfe & Chalk's root diagram; B: detail of A; C: Metcalfe & Chalk's bark diagram; D: detail of C.

#### Non-common characteristics of the roots

Comparisons of non-common characteristics of the roots are summarized in Table I. Detailed descriptions are presented below.

#### *Pfaffia glomerata*

The root is 2-2,5 cm in diameter. The transverse root sections show a uniform rhytidome, made up of a single

peridermis with 6 to 8 layers of almost tabulet phellem cells (Figure 2, H). The layers were formed by rows of thin-walled cells. The phelloderm was unstratified. Roots show 4-5 rings of collateral vascular bundles embedded in conjunctive parenchyma. Large idioblasts with numerous minute crystals of calcium oxalate are present in the conjunctive tissue (Figure 2, F).

In this species the strands of xylem and phloem are quite small, the phloem consisting of 3-4 sieve tubes only. The xylem strands contain 1-2 rows of 2-3 radially placed vessels. The fibres with small simple pits are few and shows a thick-walled (Figure 2, I).

- Macerated roots. The analysis of the material yielded the following constitutive elements:

- Phellem cells. In cross-sections they were rectangular with thin-walled cells and measuring 50-80 µm in length and 40 µm in wide. In the superficial view they are polygonal, attaining a diameter of 50 µm (Figure 2, H, G).
- Conjunctive parenchyma. It showed polygonal cells. The average dimensions of parenchyma cells are 170 µm in length and 50 µm in wide (Figure 2, E).
- Vessel. The vessels possessed alternate bordered pits with a simple perforation plate in transverse to slightly oblique end walls and measuring 100 µm in length and 50 µm in diameter (Figure 2, A, D).
- Xileme fibers. They are scanty and measuring 250-300 µm in length and 10-12 µm in diameter (Figure 2, A, C).
- Fibre-tracheid. Measuring 150 µm in length and 10-12 µm in diameter (Figure 2, B).

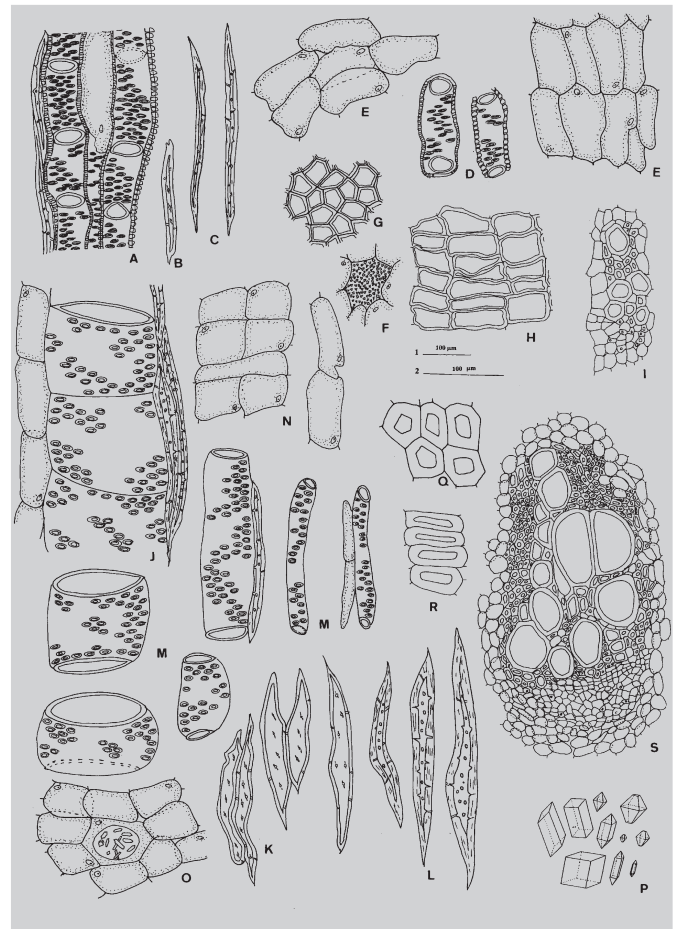
***Pfaffia paniculata***

The root is 3-4 cm in diameter. The transverse root sections show a uniform rhytidome, made up of a single peridermis with 12 to 14 layers of almost tabulet phellem cells. The layers were formed by rows of thick-walled cells (Figure 2, Q, R). The phelloderm was unstratified. Roots show 12-14 rings of collateral vascular bundles embedded in conjunctive tissue. Large idioblasts with calcium oxalate crystals of different shapes are present in the conjunctive tissue; i.e., octahedral prisms, rectangular prisms and octahedrons (Figure 2, O, P).

In this species the strands of xylem and phloem are large, the phloem consisting of 6-8 sieve tubes. The xylem strands contain 1-4 rows of 4-6 radially placed vessels. Fibbers with small simple pits are abundant (Figure 2, S).

- Macerated roots. The analysis of the material yielded the following constitutive elements:

- Phellem cells. In cross-sections they were rectangular with thick-walled cells and measuring 75 µm in length and 45 µm in wide. In the superficial view they are polygonal, attaining a diameter of 45-50 µm (Figure 2, Q, R).
- Conjunctive parenchyma. It showed elongated longitudinal cells. The average dimensions of parenchyma cells are 100 µm in length and 50 µm in wide (Figure 2, N).



**FIGURE 2 - A-S: Non-common characteristics. A-I *Pfaffia glomerata* (Spreng) Pedersen; A: Xylem, longitudinal view; B: fibre-tracheid; C: fibres; D: vessel; E: conjunctive parenchyma; F: idioblasts with numerous minute crystals calcium oxalate; G: phellem in superficial view; H: phellem in cross section; I: collateral vascular bundles; J-S: *Pfaffia paniculata* (Mart.) O. Kuntze J: Xylem, longitudinal view; K: fibre-tracheid; L: fibres; M: vessel; N: conjunctive parenchyma; O: idioblasts with calcium oxalate crystals, octahedric, rectangular prisms and octahedrons; P: crystals; Q: phellem in superficial view; R: phellem in cross section; S: collateral vascular bundles**

- c) Vessel. The vessels possessed alternate bordered pits with a simple perforation plate in transverse to slightly oblique end walls. The mean vessel member length and diameter are 40  $\mu\text{m}$  and 120  $\mu\text{m}$  respectively and others vessels measuring 165  $\mu\text{m}$  in length and 65  $\mu\text{m}$  in diameter (Figure 2, J, M).
- d) Xileme fibres. They are very abundant, thick-walled, measuring 350  $\mu\text{m}$  in length and 25  $\mu\text{m}$  in diameter (Figure 2, J, L).
- e) Fibre-tracheid. Thin-walled, they measured 200  $\mu\text{m}$  in length and 35  $\mu\text{m}$  in diameter (Figure 2, K).

The examined species differ in morphologic and anatomic features, as shown in Table I.

### Chemical and biological data

Ethanollic extracts from *P. glomerata* and *P. paniculata* present different TLC profiles at the chromatographic conditions used. It was possible to identify the presence of ecdysterone in *P. glomerata* extract as already described in the literature (Shiobara *et al.*, 1993a), but not

in *P. paniculata* extract. It seems that this compound could be a good marker for the differentiation of both species. The other substances used in TLC were not good references as both species presented spots with similar Rf and color.

Regarding to biological assays, ethanolic extract from *P. glomerata* did not present neither antiproliferative, antiviral, antifungal nor MAO inhibitory activities. Nevertheless, it is interesting to note that its cytotoxicity is very low ( $\text{IC}_{50} > 2,000 \mu\text{g/mL}$ ).

From results reported here, we could observe that roots from *P. glomerata* and *P. paniculata* differ in their botanical parameters and TLC profile, being both characteristics useful for avoiding adulterations.

Regarding results obtained in biological assays, *P. glomerata* showed to be inactive in all the activities tested, such as antifungal, antiviral, antitumoural and MAO inhibition. Interesting enough, our studies demonstrated that this species is not cytotoxic.

Finally, careful evaluation of the available scientific data for *Pfaffia* species allow us to consider that more studies are necessary to provide chemical, pharmaco-

**TABLE I** - Non-common features of *Pfaffia glomerata* and *Pfaffia paniculata*

<i>Pfaffia glomerata</i>	<i>Pfaffia paniculata</i>
<b>Exomorphology</b> The root is 2-2.5 cm in diameter	<b>Exomorphology</b> The root is 3-4 cm in diameter
<b>Anatomy</b> <ul style="list-style-type: none"> <li>• A single peridermis with 6 to 8 layers, thin-walled cells.</li> <li>• 4-5 rings of small collateral vascular bundles embedded in conjunctive tissue.</li> <li>• The xylem strands contain 1-2 rows of 2-4 radially placed vessels.</li> </ul>	<b>Anatomy</b> <ul style="list-style-type: none"> <li>• A single peridermis with 12 to 14 layers, thick-walled cells.</li> <li>• 12-14 rings of large collateral vascular bundles embedded in conjunctive tissue.</li> <li>• The xylem strands contain 1-4 rows of 4-6 radially placed vessels.</li> </ul>
<b>Macerate</b> <ul style="list-style-type: none"> <li>• Phellem cells: Measuring 50-80 <math>\mu\text{m}</math> in length and 40 <math>\mu\text{m}</math> in wide.</li> <li>• Conjunctive parenchyma: Cell size 100 <math>\mu\text{m}</math> in length and 50 <math>\mu\text{m}</math> in wide. Large idioblasts with numerous minute crystals of calcium oxalate.</li> <li>• Vessel: 100 <math>\mu\text{m}</math> in length and 100 <math>\mu\text{m}</math> in diameter.</li> <li>• Fibres: 250-300 <math>\mu\text{m}</math> in length and 10-12 <math>\mu\text{m}</math> in diameter.</li> <li>• Fibre-tracheid: 150-300 <math>\mu\text{m}</math> in length and 10-12 <math>\mu\text{m}</math> in diameter</li> </ul>	<b>Macerate</b> <ul style="list-style-type: none"> <li>• Phellem cells: Measuring 75 <math>\mu\text{m}</math> in length and 45 <math>\mu\text{m}</math> in wide.</li> <li>• Conjunctive parenchyma: Cell size 100 <math>\mu\text{m}</math> in length and 50 <math>\mu\text{m}</math> in wide. Large idioblasts with calcium oxalate crystals, octahedric, rectangular prisms and octahedrons.</li> <li>• Vessel: 40 <math>\mu\text{m}</math> in length and 120 <math>\mu\text{m}</math> in diameter; 165 <math>\mu\text{m}</math> in length and 65 <math>\mu\text{m}</math> in diameter</li> <li>• Fibres: thick-walled, 350 <math>\mu\text{m}</math> in length and 25 <math>\mu\text{m}</math> in diameter.</li> <li>• Fibre-traqueid: Thin-walled, 200 <math>\mu\text{m}</math> in length and 35 <math>\mu\text{m}</math> in diameter.</li> </ul>

gical and technological basis for their therapeutic use. This is really important taking into account that several phytopharmaceutical preparations containing *P. glomerata* or *P. paniculata* are available in Brazilian market without definitive proofs of their efficacy and safety. Even if eventual claim for traditional use of these preparations is considered, there are not consistent ethnobotanic reports on *P. glomerata* and *P. paniculata*. Furthermore, it is necessary to point out that the quality criteria for both - raw materials and phytopharmaceutical preparations - are still not well established. This study intends to have contributed specially toward the latest question.

## RESUMO

### Características botânicas (morfológicas, micrográficas), químicas e farmacológicas de espécies de *Pfaffia* (Amaranthaceae) nativas do Sul do Brasil

Alguns parâmetros são apresentados para o controle de qualidade de raízes de *Pfaffia glomerata* e *Pfaffia paniculata*, utilizando suas características botânicas e químicas. Realizou-se, também, um screening farmacológico de *P. glomerata* direcionado às atividades relacionadas com seu uso como “tônico”. O extrato etanólico das raízes de *P. glomerata* não apresentou atividade antiviral, antiproliferativa, antifúngica ou atividade inibitória de monoaminoxidases. A avaliação da citotoxicidade determinou que a  $CI_{50}$  é  $> 2.000 \mu\text{g/mL}$ . As principais características morfológicas e micrográficas das raízes de *P. glomerata* e *P. paniculata* descritas permitem auxiliar na sua identificação segura.

UNITERMOS: *Pfaffia glomerata*. *Pfaffia paniculata*. Ginseng brasileiro. Micrografia. Controle de qualidade.

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