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Chemical composition fluctuations in roots of *Plumbago scandens* L. in relation to floral development

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ABSTRACT

Plumbago scandens L. is a Brazilian tropical/subtropical species that occurs along the coast. Chemically it is mainly represented by naphthoquinones, flavonoids, terpenoids and steroids. The aim of the present work is to study quantitative changes in the root metabolic production of *Plumbago scandens* during different physiologic developmental stages relative to floration. The results indicated the presence of four substances in the extracts: plumbagin, epi-isoshinanolone, palmitic acid and sitosterol, independent on developmental stage. The naphthoquinone plumbagin has always showed to be the major component of all extracts. Naphthoquinones exhibited their highest content during floration, while the content of the two others components decreased during this stage, revealing an inverse profile. The chemical composition changed depending on the plant requirements.

Key words: GC/MS, plant physiology, Plumbaginaceae, secondary metabolites.

INTRODUCTION

Plumbago scandens L. belongs to the family Plumbaginaceae, order Plumbaginales, superorder Plumbaginanae, according to Dahlgren 1989. In this system of classification, Plumbaginales is positioned in the vicinity of Caryophyllales and Polygonales, suggesting possible affinities. According to the system proposed by the Angiosperm Phylogeny Group, the family Plumbaginaceae is placed with Polygonaceae, in the order Caryophyllales (APGIII 2009). Although they are systems that use different tools, in both cases the relationship with Caryophyllaceae and Polygonaceae was pointed.

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Plumbago scandens is a subshrub with white flowers quite widespread in Brazil, being found along the coast. It is a native species found in a typical vegetation of "restinga", which is characterized by high luminous intensity, sandy soil and water restriction. This plant has already disappeared in many places due to human's activity. It can also be found in Southerm Florida, Texas and Arizona, Mexico, Central America, the west Indies and South America (Verhoek-Williams 1970). Popularly this species is known as "louco" since their leaves are used as nape compresses in people with mental disorders in order to calm them down (Braga 1960 *apud* Lorenzi and Matos 2002). *P. scandens* is also known as "caataia", "caapomonga", "folha-de-louro", "erva-do-diabo", among others (Lorenzi and Matos 2002). According to Mors et al. 2000 *apud* Lorenzi and Matos

2002, several uses are attributed to preparations obtained from roots, such as purgative and local anesthetic. It is also used as decoction or infusion to soothe toothaches and earaches, as well as to reduce joints inflammation. There are few studies in the literature about the chemistry of the species. However, there is a record production of naphthoquinones, steroids and flavonoids (Bhattacharyya and Carvalho 1986, Paiva et al. 2004, 2002). Among the isolated compounds, the naphthoquinone plumbagin deserves attention by the description of numerous pharmacological activities, such as antimicrobial (Paiva et al. 2003), anti-tumoral (Devi et al. 1999, Lin et al. 2003), trypanocidal (Sepúlveda-Bozza and Cassels 1996) and antimalarial (Suraveratum et al. 2000), among others. This naphthoquinone is mostly found in roots of several species of the genus *Plumbago* (Van der Vijver 1972).

Organic compounds make up the food for the cell and the structural components of the wall and the protoplasm, besides other special compounds, such as hormones, pigments and enzymes (Weier et al. 1982). Depending on the plant physiological stage, these compounds show changes in their contents.

The production of secondary metabolites is the result of complex interactions among biosynthesis, transport, reservoir and degradation. Each process is controlled by genes, hence it will be influenced by three main factors: hereditarity, ontogenesis and surroundings (Robbers et al. 1996).

This work aimed to verify the micromolecular composition changes in roots of *Plumbago scandens* L. during the floral development in order to analyze the influence of physiological stages in chemical production.

MATERIALS AND METHODS

Solvents

The solvents used (chloroform and ethyl acetate) were PA grade.

Plant Material

Plumbago scandens L. was collected at Fundação Oswaldo Cruz campus, Rio de Janeiro, Brazil. A voucher of this plant was deposited at the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro Herbarium (RB) under the number 340.340.

Extracts Preparation

The roots of *P. scandens* were collected in 2001 in different periods (before, during and after floral development, which corresponded to the months of March, April and May, respectively). The dried powdered roots of each plant material (9,5g) were submitted to a dynamic extraction with 300 ml of chloroform (three extractions of 100ml). All extracts were evaporated to dryness under reduced pressure.

Instruments

Instrumentation consisted of an Agillent Technologies gas chromatograph model 6890N equipped with a mass selective detector, model 5973, and an automatic injector model 5683, as well as a capilar column HP-5MS (5% phenyl, 95% methyl syloxan), 30 m $\times$ 0,25 mm $\times$ 0,25 μm. Data acquisition was performed by HP Chemistation Data Acquisition Software.

Sample Preparation

A portion of each crude chloroform extract (2,0 mg) was dissolved in ethyl acetate (1 ml) and injected into a gas chromatograph coupled with a mass spectrometer. The injections were performed in triplicates.

Chromatographic Conditions

The following conditions were used: helium as the carrier gas, mass detector, detector temperature = 280°C, injector temperature = 270°C, flow rate 1,0 ml/min, split of 1:20, injection volume = 1,0 μl, initial temperature = 120°C and oven programme from 5°C/min to 290°C followed by an isotherm period of 20 min.

RESULTS AND DISCUSSION

The three extracts revealed interesting aspects. The results indicated a trend towards an increased production of extracts on the basis of flowering. The major extract yield was obtained after the floral development. However, the highest increase of extract production to achieve the blooming was up to 23% (Fig. 1).

The quantitative determination of chemical constituents in the extracts was evaluated by a gas chromatography coupled to a mass spectrometry. It was verified the presence of four compounds in all extracts (Fig. 2).

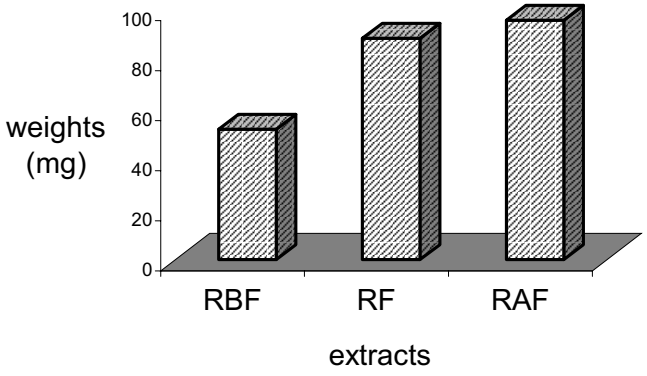


Fig. 1 – Weights of the crude chloroform extracts from roots of *Plumbago scandens* collected in different development stages. RBF: roots before floration; RF: roots during floration; RAF: roots after floration.

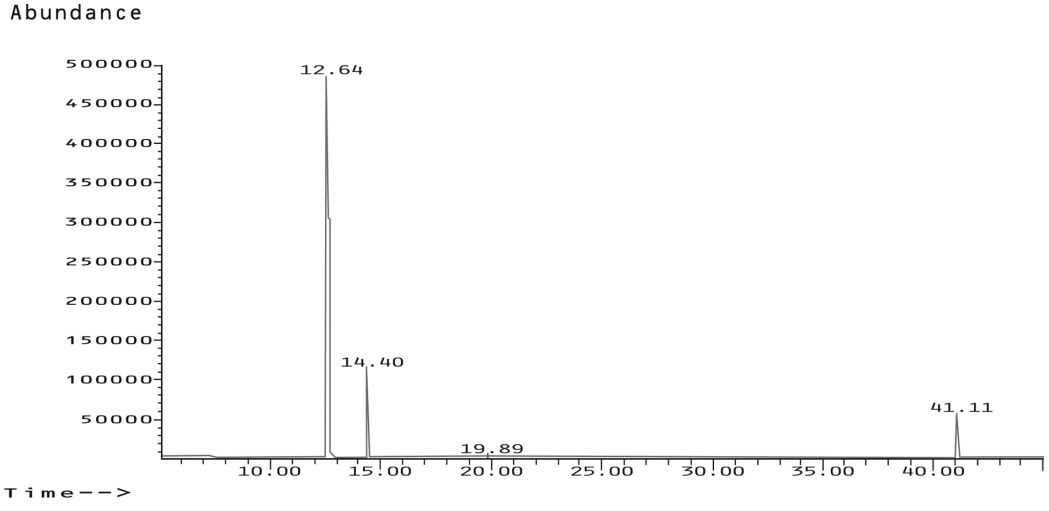
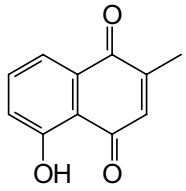


Fig. 2 – Chromatogram of the crude chloroform extracts from roots of *Plumbago scandens* after floration.

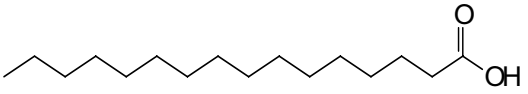
The analysis of the spectral data allowed to identify the compounds as plumbagin **(1)**, epi-isoshinanolone **(2)**, palmitic acid **(3)** and sitosterol **(4)** (Table I). The naphthoquinone plumbagin has always been the major compound present in the root extracts. The lowest level of this substance in roots was observed before the floral development. During floration, the content of the naphthoquinone plumbagin had a great increase, reaching its highest level. Previous studies (Paiva unplublished data) showed that, in *P. scandens*, this naphthoquinone is found predominantly on the roots, with low concentrations in aerial parts. In other species of the genus *Plumbago*, such as *P. rosea*, the literature data also point to the accumulation of plumbagin in roots (Panichayupakaranant and Tewtrakul 2002). A study carried out by Verma et al. 2002, revealed the potentialities of the hairy root cultures of *P. zeylanica* for the production of plumbagin. However, it is important to point out the work of Kitanov and Pashankov (1994), which showed that the flowers of the inflorescences of *P. europaea* have high concentrations of this substance.

It may function in the plant chemical defense against pathogens. During floral development, the plant may be more susceptible to microorganisms attack, once its metabolism can be involved in the transition of the vegetative caulinar apex into a reproductive apex. Moreover, the roots are always exposed to different soil microorganisms, which may explain the accumulation of defense compounds such as the naphthoquinone plumbagin.

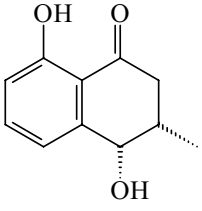
The other naphthoquinone, epi-isoshinanolone,



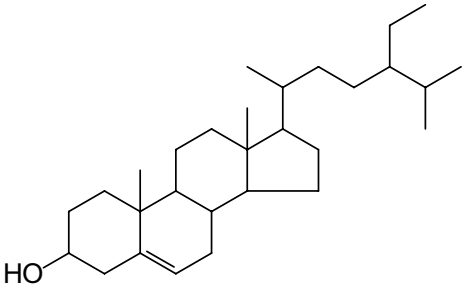
Plumbagin (1)



Palmitic acid (3)



Epi-isoshinanolone (2)



Sitosterol (4)

TABLE I
Compounds characterized in *Plumbago scandens* root extracts during different physiologic developmental stages.

	Area				
Compound	RT (min)	Molecular weight	RBF	RF	RAF
Plumbagin	12.60	188	30.369.438	137.163.771	102.492.599
Isoshinanolone	14.40	192	284.547	1.511.758	1.491.249
Palmitic acid	19.90	256	707.569	140.042	67.009
Sitosterol	41.13	414	4.813.528	927.970	634.677

RBF: roots before floration; RF: roots during floration; RAF: roots after floration; RT (min): retention time (minutes).

functioned in the same way as plumbagin, showing the major yield during the floral development.

The analysis of other components showed a great variation on their contents. Sitosterol and palmitic acid levels demonstrated the same profile, decreasing during the floral development. Root tips are a metabolically active tissue with a high energy demand, and the role of fatty acid β-oxidation in this tissue is likely to be in membrane lipid turnover (Graham and Eastmond 2002). *P. scandens* roots do not realize photosynthesis, and the energy demand is higher than the arrival of products (carbohydrates) from the aerial parts, especially during the floral development. It could be said that the roots represent a physiological drain since they demand energy and food for their growth and maintenance.

According to Graham and Eastmond (2002), under carbohydrate starvation conditions, the respiratory demand for C-skeletons derived from fatty acid β-oxidation is expected to increase the triggering induction of the breakdown pathway. The decrease in palmitic acid and sitosterol level suggests their consumption for energy supply and for the construction of plasma membranes in the new cells generated by the activity of the root apical meristem.

Moreover, cellular sterol content and composition may also play a regulatory role in important events in the life cycle of higher plants. The analysis of the sterol content and the composition of developing floral apices from *Lolium temulentum* confirmed that the sterol content decreased during the inductive period, and a much

greater amount of cholesterol was present in the apex, compared to other parts of the plant. It was suggested that such changes in sterol composition mediate membrane permeability, and this might affect the transport of metabolites during evocation (Hobbs et al. 1996).

CONCLUSION

Root chemical composition of *Plumbago scandens* is affected by the plant physiological stage. Plant requirements, depending on the stage, can vary, and this fact influences the chemical production. The increase in the content of defense compounds such as naphthoquinones during the floral development may protect the plant during a susceptible period. The content of steroids and fatty acids in roots decreased in the same stage, suggesting their consumption and a declined supply of organic material to the roots.

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RESUMO

Plumbago scandens L. é uma espécie brasileira tropical/subtropical que ocorre ao longo da costa. Quimicamente, é principalmente representada por naftoquinonas, flavonóides, terpenóides e esteróides. O objetivo do presente trabalho é estudar mudanças quantitativas da produção metabólica nas raízes de *Plumbago scandens* durante diferentes estágios de desenvolvimento fisiológico, relativos à floração. Os resultados indicaram a presença de quatro substâncias nos extratos: plumbagina, epi-isoshinanolona, ácido palmítico e sitosterol, independente do estágio de desenvolvimento. A naftoquinona plumbagina tem sempre mostrado ser o componente majoritário de todos os extratos. Naftoquinonas exibiram seus maiores conteúdos durante a floração, enquanto o conteúdo dos dois outros componentes decresceu durante este estágio, revelando um perfil inverso. A composição química modificou dependendo das necessidades da planta

Palavras-chave: CG/EM, fisiologia vegetal, Plumbaginaceae, metabólitos secundários.

REFERENCES

APGIII. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot J Linn Soc 161(2): 105–121.

BHATTACHARYYA J AND CARVALHO VR. 1986. Epi-isoshinanolone from *Plumbago scandens*. Phytochem 25(3): 764–765.

BRAGA RA. 1960. Plantas do Nordeste, especialmente do Ceará, 2ª ed., Fortaleza: Impr. Oficial, 540 p. *apud* Lorenzi H and Matos FJA. 2002. Plantas medicinais no Brasil: nativas e exóticas, São Paulo: Nova Odessa, Instituto Plantarum, 512 p.

DAHLGREN G. 1989. An updated angiosperm classification. Bot J Linn Soc 100: 197–203.

DEVI PU, SOLOMON FE AND SHARADA AC. 1999. Plumbagin, A Plant Naphthoquinone with Antitumor and Radiomodifying Properties. Pharm Biol 37(3): 231–236.

GRAHAM IA AND EASTMOND PJ. 2002. Pathways of straight and branched chain fatty acid catabolism in higher plants. Prog Lipid Res 41: 156–181.

HOBBS DH, HUME JH, ROLPH CE AND COOKE DT. 1996. Changes in lipid composition during floral development of *Brassica campestris*. Phytochem 42(2): 335–339.

KITANOV GM AND PASHANKOV PP. 1994. Quantitative investigation on the dynamics in *Plumbago europaea* L. Roots and herb by HPLC. Pharmazie 49: 462.

LIN LC, YANG LL AND CHOU CJ. 2003. Cytotoxic naphthoquinones and plumbagic acid glucosides from *Plumbago zeylanica*. Phytochem 62(4): 619–622.

LORENZI H AND MATOS FJA. 2002. Plantas medicinais no Brasil: nativas e exóticas, São Paulo: Nova Odessa, Instituto Plantarum, 512 p.

MORS WB, RIZZINI CT AND PEREIRA NA. 2000. Medicinal Plants of Brazil. Michigan: Reference Publications, 501 p. *apud* Lorenzi H and Matos FJA. 2002. Plantas medicinais no Brasil: nativas e exóticas, São Paulo: Nova Odessa, Instituto Plantarum, 512 p.

PAIVA SR, FIGUEIREDO MR, ARAGÃO TV AND KAPLAN MAC. 2003. Antimicrobial activity *in vitro* of plumbagin isolated from *Plumbago* species. Mem Inst Oswaldo Cruz 98(7): 959–961.

PAIVA SR, FONTOURA LA, MAZZEI JL, FIGUEIREDO MR AND KAPLAN MAC. 2002. Perfil cromatográfico de duas espécies de Plumbaginaceae: *Plumbago scandens* L. e *Plumbago auriculata* Lam. Quim Nova 25(5): 717–721.

PAIVA SR, LIMA LA, FIGUEIREDO MR AND KAPLAN MAC. 2004. Plumbagin quantification in roots of *Plumbago scandens* L. obtained by different extraction techniques. An Acad Bras Cienc 76: 499–504.

PANICHAYUPAKARANANT P AND TEWTRAKUL S. 2002. Plumbagin production by root cultures of *Plumbago rosea*. Electron J Biotechnol [online]. 5(3): 11–12.

ROBBERS JE, SPEEDIE MK AND TYLER VE. 1996. Pharmacognosy and pharmacobiotechnology, Baltimore: Williams & Wilkins, 337 p.

SEPÚLVEDA-BOZZA S AND CASSELS BK. 1996. Plant metabolites active against *Trypanosoma cruzi*. Planta Med 62: 98–105.

SURAVERATUM N, KRUNGKRAI SR, LEANGARAMGUL P, PRAPUNWATTANA P AND KRUNGKAI J. 2000. Purification and characterization of *Plasmodium falciparum* succinate dehydrogenase. Mol Biochem Parasitol 105(2): 215–222.

VAN DER VIJVER LM. 1972. Distribution of plumbagin in the Plumbaginaceae. Phytochem 11: 3247–3248.

VERHOEK-WILLIAMS S. 1970. Flora of Panama Part VIII Family 153 Plumbaginaceae. Ann Missouri Bot Gard 57: 55–58.

VERMA PC, SINGH D, RAHMAN L, GUPTA MM AND BANERJEE S. 2002. *In vitro*-studies in *Plumbago zeylanica*: rapid micropropagation and establishment of higher plumbagin yielding hairy root cultures. J Plant Physiol 159(5): 547–552.

WEIER TE, STOCKING CR, BARBOUR MG AND ROST TL. 1982. Botany. An introduction to plant biology, 6th ed., New York: J Wiley & Sons, USA, 720 p.