Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v38i4.30567

Effect of environmental and phenological factors on the antimicrobial activity of *Cochlospermum regium* (Schrank) Pilg. roots

Marielle Cascaes Inácio[1], Tiago Antunes Paz[2], Bianca Waléria Bertoni[3] and Ana Maria Soares Pereira[3*]

*[1]Departamento de Produção Vegetal, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil. [2]Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, Departamento de Química Orgânica, Instituto de Química, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil. [3]Departamento de Biotecnologia Vegetal, Universidade de Ribeirão Preto, Av. Costábile Romano, 2201, 14096-900, Ribeirânia, Ribeirão Preto, São Paulo, Brazil. *Author for correspondence. E-mail: apereira@unaerp.br*

ABSTRACT. *Cochlospermum regium* (Bixaceae) is a medicinal plant species native to the Brazilian savannah, known as the Cerrado. Local communities use its roots to treat infections of the female reproductive system. This study evaluated how seasonality, phenological stage, geographical location, plant age, and plant cultivation substrate affect the antimicrobial activity of *Cochlospermum regium* roots against *Candida albicans*. Although the plant displayed antimicrobial action in all the assayed conditions, the roots collected during fall and winter inhibited *C. albicans* the most effectively. The geographical location in which the plant material was found did not influence its biological response. Plant age and cultivation substrate did impact the biological response; those grown in sand showed fungicidal activity, while those grown in Cerrado soil fertilized with cow dung showed fungistatic activity during certain periods. The inner bark and core of the root were the main structures underlying the antimicrobial activity of *Cochlospermum regium*.

Keywords: Bixaceae, STAMP (Screening Test for Antibiotics in Medicinal Plants), *Candida albicans*, Cerrado, algodãozinho-do-campo.

Efeito de fatores ambientais e fenológicos sobre a atividade antimicrobiana de raízes de *Cochlospermum regium* (Schrank) Pilg.

RESUMO. *Cochlospermum regium* (Bixaceae) é uma espécie de planta medicinal nativa do Cerrado. As comunidades locais usam suas raízes, principalmente, para o tratamento de infecções do sistema reprodutor feminino. Este trabalho avaliou como a sazonalidade, o estádio fenológico, a localização geográfica, a idade da planta e o substrato de cultivo afeta a atividade antimicrobiana de raízes de *C. regium* contra o microrganismo patogênico *Candida albicans*. Embora a planta tenha exibido ação antimicrobiana em todas as condições avaliadas, as raízes coletadas durante o outono e inverno foram mais eficazes contra *C. albicans*. A localização geográfica, a qual o material vegetal se encontrara, não influenciou em sua resposta biológica. A idade da planta e o substrato de cultivo também influenciaram na resposta biológica, sendo que as cultivadas em areia apresentaram atividade fungicida enquanto que as cultivadas em solo de Cerrado+esterco apresentaram atividade fungistática nos períodos avaliados. A entrecasca e o cerne da raiz foram as principais estruturas responsáveis pela atividade antimicrobiana de *Cochlospermum regium*.

Palavras-chave: Bixaceae, STAMP (Teste de triagem de antimicrobianos em plantas medicinais), *Candida albicans*, Cerrado, algodãozinho-do-campo.

Introduction

Cochlospermum regium (Schrank) Pilg., Bixaceae, is a medicinal plant native to the Brazilian savannah, or the Cerrado. According to ethnopharmacological data, its roots are applied to treat infections of the female reproductive system (Nunes, Silva, & Rezende, 2003; Souza & Felfili, 2006; Moreira & Guarim-Neto, 2009). *Cochlospermum regium* has been widely investigated for two main reasons: its medicinal potential and its existence in the Cerrado, a hotspot biome that occupies 21% of Brazilian territory and contains 4,400 endemic plant species (Zachos & Habel, 2011).

Researchers have already isolated and identified several substances in *Cochlospermum regium* roots, e.g., dihydrokaempherol-3-O-β-glucopyranoside (Lima, Castro, Mello, Siqueira, & Kassab, 1996; Sólon et al., 2012), β-selinene, (Honda, Brum, Hess, Cruz, & Moretto, 1997), ellagic acid, gallic acid, dihydrokaempherol, dihydrokaempherol-3-O-β-(6"-galloyl)-glucopyranoside, pinoresinol, excelsine,

and the triacylbenzenes cochlospermines A and B (Sólon et al., 2012). However, the compound or compounds that underlie the antimicrobial activity described in ethnopharmacological reviews remain unknown. In addition, scientific papers have failed to agree on the antimicrobial efficiency of root extracts as assessed by *in vitro* tests (Oliveira, Siqueira, Souza, & Rezende, 1996; Sólon et al., 2012), besides the studies published in theses and at conferences. Divergences may result from the environmental and phenological factors at play at the time of plant collection. Climate, seasons of the year, phenological stage, genetic load, temperature, altitude, cultivation conditions and humidity, among other factors, can significantly affect the quality and/or quantity of bioactive compounds (Szakiel, Pączkowski, & Henry, 2011; Pavarini, Pavarini, Niehues, & Lopes, 2012).

In this context, this work aimed to investigate how seasonality, phenological stage, geographical location, plant cultivation substrate, and plant age influence the antimicrobial activity of *Cochlospermum regium* roots against the pathogenic microorganism *Candida albicans*. In addition, we aimed to investigate the specific root structure (bark, inner bark and core) responsible for the fungicidal effect.

Material and methods

Plant material and sample preparation

To conduct the antimicrobial assays, roots from 58 *Cochlospermum regium* specimens were collected in five populations inhabiting different states in the Brazilian Cerrado: (1) São Paulo, in the municipalities of Altinópolis (Latitude: 21°03'26.8", Longitude: 47°29'27.4", Altitude: 607 m; 24 specimens) and Rifaina (Latitude: 20°04'49.8", Longitude: 47°25'48.8", Altitude: 612 m; 12 specimens); (2) Minas Gerais, in the municipality of Sacramento (Latitude: 19°58'53.6", Longitude: 47°24'20.7", Altitude: 645 m; 12 specimens); (3) Goiás, in the municipality of Campo Alegre de Goiás (Latitude: 17°49'14.7", Longitude: 47°46'13.4", Altitude: 953 m; six specimens); and (4) Mato Grosso do Sul, in the municipality of Terenos (Latitude: 20°25'31.2", Longitude: 55°02'44.6", Altitude: 437 m; four specimens). Permission to collect specimens from natural habitats was provided by Conselho de Gestão do Patrimônio Genético (the Council for Genetic Heritage Management, CGEN – Process number 02001.05103/2011-42). Soil samples were collected for each specimen and submitted for chemical analysis.

In the research laboratory, the roots were washed with detergent, running water, and they were brushed. Next, they were separated into bark, inner bark, and core. They were then dried in a circulating air oven at 45ºC for 48h, milled in a knife mill, and sieved (48 mesh). Whole roots were also used in the assays. The activities of *C. regium* root bark, inner root bark, root core, and whole root were compared in all of the experiments, except for the experiment conducted in a greenhouse, which evaluated the effect of age and substrate in the antimicrobial activity of roots.

The STAMP (Screening Test for Antibiotics in Medicinal Plants) (Inácio et al., 2013) method and the microorganism *Candida albicans* ATCC 10231 were employed to evaluate the antimicrobial activity. This method uses powdered plant materials instead of extracts. The raw plant material was dried in an oven at 45ºC under circulating air, ground in a knife mill and sieved to standardize the particle size (48 mesh). The mass of dry powdered plant material deposited in each well (28 mg) was calculated from its density and the volume of the well.

A totally random statistical design based on four specimens was used to assess the influence of geographical location, and six specimens were used to assess the other parameters. Statistical analyses were conducted using a Tukey's test with 0.5% probability. The program SISVAR aided the statistical calculations (Ferreira, 2011).

Effect of phenological stage on the antimicrobial activity of *C. regium* roots

To evaluate how the phenological stage affects the antimicrobial activity of *C. regium* roots, the roots of specimens growing in the municipalities of Altinópolis (São Paulo State), Rifaina (São Paulo State) and Sacramento (Minas Gerais State) were collected in the morning during the vegetative/pre-flowering and flowering stage. Six specimens were assessed for each phenological stage and population.

Effect of seasonality on the antimicrobial activity of *C. regium* roots

To examine how seasonality impacts the antimicrobial activity of *C. regium* roots, specimens were always collected at the end of summer (March), spring (December), fall (June), and winter (September) in the municipality of Altinópolis (São Paulo State), also in the morning. Six specimens were collected at the end of each season.

Effect of geographical location on the antimicrobial activity of *C. regium* roots

To assess how geographical location influences the antimicrobial action of *C. regium* roots, the roots of specimens growing in the municipalities of Altinópolis (São Paulo State), Rifaina (São Paulo State), Sacramento (Minas Gerais State), Campo Alegre de Goiás (Goiás State) and Terenos (Mato Grosso do Sul State) were collected during the vegetative stage. Four specimens were assayed for each population.

Meteorological data (temperature, relative humidity and rainfall) on the *Cochlospermum regium* collection months and location were obtained from automated stations of the Instituto Nacional de Metereologia (Brazilian Meteorology Institute, INMET, http://www.inmet.gov.br).

Effect of plant age and cultivation substrate on the antimicrobial activity of *C. regium* roots

To assess how plant age and cultivation substrate affect the antimicrobial activity of *C. regium* roots, plants were cultivated in a greenhouse in Ribeirão Preto city (São Paulo State - Latitude: 21°10'40", Longitude: 47°48'36", Altitude: 546m); seeds were collected in the same municipalities as the roots. The seeds were grouped into a single lot, which was used for cultivation. Two cultivation substrates were employed: 1) Cerrado soil (oxisol soil) mixed with cow dung (1:1) and 2) sand, which represents the extreme concentration of soil nutrients. The plants were watered manually twice a day. Analyses were performed on plants aged two, four, six, nine, and twelve months. To perform the experiments, thirty whole plant roots collected at different ages were grouped into a single lot of ten roots each, and the antimicrobial tests were carried out in triplicate.

Effect of soil constituents on the antimicrobial activity of *C. regium* roots

A simple correlation helped to evaluate how soil constituents influenced the antimicrobial action of *C. regium* roots. Analysis of the main components (Principal Components Analysis - PCA) was also accomplished with the aid of the program Genes VS 2009.7.0 (Cruz, 2006). The antimicrobial activity data used in this analysis were obtained during the assay that evaluated the effect of geographical location. The analysis of the main components included 17 variables: biological activity, organic matter (OM), pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S-SO_4), total bases (TB), potential acidity (H + Al), cation exchange capacity (CEC), bases saturation (V), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Results and discussion

Effect of phenological stage on the antimicrobial activity of *C. regium* roots

The phenological stage did not elicit a standard antimicrobial response (Table 1). For the plants collected in Altinópolis, whole roots and inner root barks of specimens in the vegetative/pre-flowering stage displayed higher antimicrobial activity compared with specimens in the flowering stage. Concerning the plants collected in Rifaina, specimens in different phenological stages (vegetative and flowering) did not differ significantly in terms of antimicrobial action. As for the roots collected in Sacramento, their response contrasted with the response of the specimens collected in Altinópolis: all of the root structures, as well as the whole root collected during the flowering stage, exhibited higher antimicrobial activity compared with those collected in the vegetative stage.

Table 1. Effect of phenological stage on the antimicrobial activity of *Cochlospermum regium* whole roots as well as root bark, inner bark, and core collected in the municipalities of Altinópolis (State of São Paulo), Rifaina (State of São Paulo), and Sacramento (State of Minas Gerais) against *Candida albicans* ATCC 10231.

Phenological stage	Diameter of the inhibition zones (mm)			
	Altinópolis			
	Root bark	Root inner bark	Root core	Whole roots
Vegetative/pre-flowering	4.28aB	26.06aA	22.72aA	24.06aA
Flowering	0.00aB	18.66bA	19.22aA	19.00bA
	Rifaina			
Vegetative/pre-flowering	0.00bB	19.28aA	18.39aA	19.00aA
Flowering	10.61aA	19.33aA	14.44aA	16.83aA
	Sacramento			
Vegetative/pre-flowering	7.00bB	17.50bA	14.89bA	16.89bA
Flowering	24.00aA	27.56aA	20.55aA	23.89aA

The means followed by the same small letter in the column and the same capital letter in the row do not differ statistically on the basis of Tukey's test at 0.5% probability.

In plants, bioactive metabolites vary depending on the phenological stage (Sartorelli, Marquioreto, Amaral, Lima, & Moreno, 2007; Çıtak, Radušienė, Janulis, & Ivanauskas, 2007; Ebrahimi, Hadian, Mirjalili, Sonboli, Yousefzadi, 2008). In the case of *C. regium* roots, as a whole or in parts (Figure 1A and B), the phenological stage did not elicit a standard response for the specimens collected in different geographical locations. Therefore, geographical location may interfere with, but not determine, the biological response.

Effect of seasonality on the antimicrobial activity of *C. regium* roots

The different seasons interfered with the antimicrobial response of the whole root and of the root bark, inner bark, and core (Table 2). In all

samples, with the exception of the root bark, fall and winter favored antimicrobial activity; winter elicited the most action in the root bark compared with the other seasons. With respect to the root bark, there was an accumulation of biologically active metabolites over seasons: in the spring, the root bark was not active, but this activity started increasing in the summer and fall; it peaked in the winter (14.32 mm – Table 2).

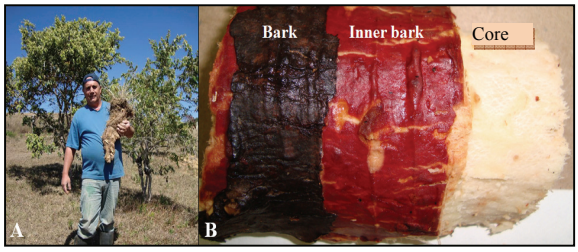

Figure 1. *Cochlospermum regium* (A) whole root and (B) root parts: bark, inner bark, and core.

Table 2. Effect of seasonality on the antimicrobial activity of *Cochlospermum regium* whole roots as well as root bark, inner bark, and core collected in the municipality of Altinópolis (State of São Paulo) against *Candida albicans* ATCC 10231.

Season	Diameter of the inhibition zones (mm)			
	Root bark	Root inner bark	Root core	Whole roots
Spring	0.00bB	18.66bA	19.22aA	19.00bA
Summer	1.83bB	16.00bA	15.05bA	16.33bA
Fall	4.28bB	26.06aA	22.72aA	24.06aA
Winter	14.32aA	24.50aA	19.11aA	21.95aA

The means followed by the same small letter in the column and the same capital letter in the row do not differ statistically on the basis of Tukey's test at 0.5% probability.

Two seasons occur in the Cerrado: the dry season (fall and winter) and the rainy season (spring and summer); temperatures do not vary significantly. At the start of fall, *C. regium* leaves begin the process of senescence; in winter, almost 100% of the plants are deciduous. The plant experiences a higher level of stress during fall and winter, which may be observed when the humidity is low and leaves are absent. Consequently, the antimicrobial compounds migrate to the root bark to protect the root. The higher antimicrobial action verified in other root parts during fall and winter demonstrates that secondary metabolites accumulate in these parts during the drier seasons. In general, the substances produced in the secondary metabolism are more concentrated when plants are under drought stress (Selmar & Kleinwaechter, 2013). One explanation for this finding relates to the stoma closure in response to this stress. When this event happens, no CO_2 absorption occurs, and much of the $NADPH^+H^+$ that the Calvin cycle ceases to consume participates in the synthesis of highly reduced compounds such as isoprenoids, simple and complex phenols, and alkaloids (Selmar & Kleinwaechter, 2013).

During the dry period, leaves are absent in *C. regium*, so CO_2 absorption all but ceases to occur. As a result, active compounds accumulate in the roots. Moreover, although the active compound has not been identified yet, *C. regium* and other species of this genus are known to be rich in flavonoids, which are complex phenols (Sólon, Brandão, & Siqueira, 2009; Sólon et al., 2012) that can protect the plant during the dry season (Ramakrishna & Ravishankar, 2011).

This work has verified that both the whole root and the root parts exhibit similar antimicrobial activity regardless of the season. When considering the fall and winter, the seasons that accounted for most of the observed antimicrobial activity, we find that, statistically, the activity in the whole root was similar to that of other structures (Table 2). Thus, there is no need for the local population to separate the root parts before using the plant.

Effect of geographical location on the antimicrobial activity of *C. regium* roots

C. regium populations located in different geographical positions displayed similar antimicrobial activity (Table 3). The fungicidal activity was statistically similar between the different locations and different structures (except for root bark of plants collected in Rifaina and Altinópolis). Therefore, the active compounds present in *C. regium* are constitutive, and the environmental factors assessed in this work do not influence the antimicrobial action. Again, this assay revealed that it is best to use the whole root as a phytotherapic medicine.

It is noteworthy that the root core, inner root bark, and root bark represent 57.11% (±7.23), 26.82% (±4.64), and 16.07% (±6.87) of the root dry mass, respectively. Judging from the data obtained in the present work, the use of an isolated root part would lead to a great loss of active material.

Meteorological data from the time of plant collection that was obtained from Instituto Nacional de Metereologia (Brazilian Meteorology Institute, INMET) verified that temperature and relative humidity varied. The monthly averages of temperature and relative humidity varied the most in Terenos (State of Mato Grosso do Sul) and Campo Alegre do Goiás (State of Goiás), respectively (Table 4). Nonetheless, all of the roots collected in these areas acted as antimicrobials, which reinforced the notion that the active compounds are constitutive.

Table 3. Effect of geographical location on the antimicrobial activity of *Cochlospermum regium* whole roots as well as root bark, inner bark, and core collected in the municipalities of Rifaina (State of São Paulo), Altinópolis (State of São Paulo), Sacramento (State of Minas Gerais), Campo Alegre do Goiás (State of Goiás) and Terenos (State of Mato Grosso do Sul) against *Candida albicans* ATCC 10231.

Geographical location (populations)	Diameter of the inhibition zones (mm)			
	Root bark	Root inner bark	Root core	Whole roots
Rifaina (SP)	0.00aB	19.50aA	18.42aA	18.83aA
Altinópolis (SP)	6.42aB	26.25aA	22.83aA	24.58aA
Sacramento (MG)	10.50aA	18.08aA	14.33aA	16.33aA
Campo Alegre (GO)	10.71aA	18.42aA	15.08aA	17.75aA
Terenos (MS)	17.25aA	19.08aA	11.58aA	18.08aA

The means followed by the same small letter in the column and the same capital letter in the row do not differ statistically on the basis of Tukey's test at 0.5% probability.

Effect of plant cultivation substrate and age on the pharmacological activity of *C. regium* roots

Roots that grew in sand and in Cerrado soil fertilized with cow dung displayed the expected medicinal effect because the soil is naturally sandy. Roots aged two and four months that grew in Cerrado soil fertilized with dung did not present any antimicrobial action; roots aged six, nine, and twelve months that grew in Cerrado soil fertilized with dung exhibited fungistatic activity (Figures 2 and 3).

In sand, the fungistatic activity was evident in the four-month-old roots. Thereafter, a fungicide effect continued until the plant was 12 months old. Hence, the active substances do exist in roots cultivated in soil fertilized with dung, but they occur at lower concentrations compared with roots grown in sand, which prevents detection of the fungicidal effect that occurred in the former case. Fungistatic activity has gained prominence in the treatment of infections because it elicits lower toxicity (Graybill, Burgess, & Hardin, 1997) and stimulates the host's immune system to act on the infection. Nevertheless, even though many studies have included results on the fungistatic activity of extracts and isolated compounds, their behavior in microorganisms and humans is not known.

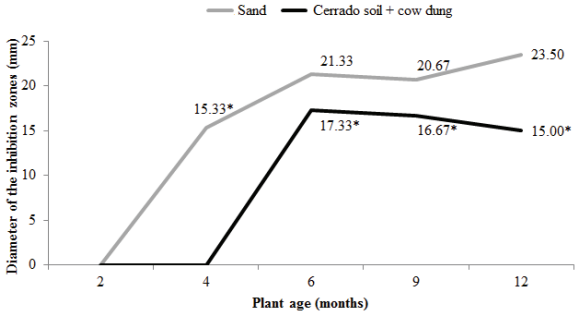

Figure 2. Antimicrobial activity of *Cochlospermum regium* roots cultivated in sand or Cerrado soil fertilized with cow dung (1:1) and collected at different ages against *Candida albicans* ATCC 10231. (*) Fungistatic activity. Values without asterisks represent fungicidal activity.

Table 4. Meteorological data on the *Cochlospermum regium* collection months and location obtained from automated stations of Instituto Nacional de Metereologia (Brazilian Meteorology Institute, INMET, http://www.inmet.gov.br). Monthly averages of temperature (ºC), relative humidity (RH%), and rainfall (mm).

Population	Date of collection	Temperature (ºC)	RH (%)	Rainfall (mm)	Weather Station
Rifaina (SP)	Jun 2011	17.87	65.63	0.93	A525 – Sacramento
Sacramento (MG)	Jun 2011	17.87	65.63	0.93	A525 – Sacramento
Altinópolis (SP)	Jun 2011	17.60	65.73	1.20	A708 – Franca
Campo Alegre do Goiás (GO)	May 2011	20.93	74.77	0.00	A033 – Pires do Rio
Terenos (MS)	Nov 2010	24.27	61.30	3.30	A702 – Campo Grande

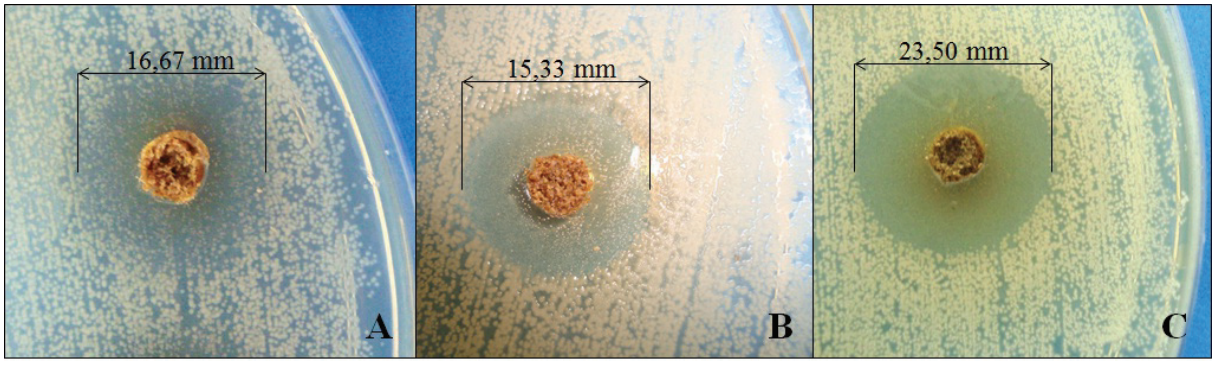

Figure 3. Average value of fungistatic activity (mm) of *Cochlospermum regium* roots cultivated in (A) Cerrado soil fertilized with cow dung (1:1) for nine months and (B) in sand for four months. (C) Fungicidal activity of *Cochlospermum regium* roots cultivated in sand for 12 months.

Notably, it is not viable to collect *C. regium* plants that are a few months old because of low yield. However, we confirmed through this experiment that the roots display antimicrobial activity at a young age. This finding was observed both in soil rich in nutrients and in nutrient-poor soil. This result shows us that the bioactive compounds from the roots are constitutive and vary only in concentration, and this finding was observed through the fungistatic activity (less accumulation of bioactive compounds) and fungicidal activity (higher accumulation of bioactive compounds).

Effect of the interaction of soil constituents on the antimicrobial activity of *C. regium* roots

The simple correlation between soil components and the antimicrobial activity of *C. regium* roots demonstrated that the poorer the soil, the more active the root was (with the exception of the bark) (Figure 4). These data corroborated the experimental results of the present work: roots cultivated in sand displayed better antimicrobial action than roots cultivated in Cerrado soil fertilized with cow dung. Plant adaptation over thousands of years explains this finding. The Cerrado biome contains soil with low levels of organic matter, low cation exchange capacity and base saturation, elevated acidity, and low nutrient availability (Faleiro & de Farias Neto, 2008). In turn, *C. regium* only occurs in the Cerrado and is well adapted to the conditions of this biome.

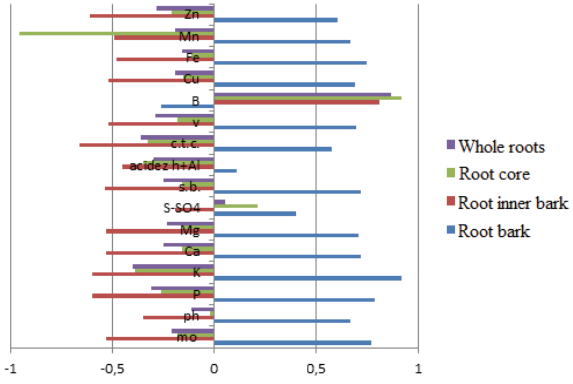

Figure 4. Correlation between antimicrobial activity and soil analysis in *Cochlospermum regium* populations.

As for the principal components, the first two components corresponded to 91.82% of the total variation. The largest loads occurred for the variables of biological activity and potassium (K) in the first and second components, respectively. Hence, these variables contributed the most to the observed variability.

A graphic dispersion of the five *C. region* populations with respect to the first two main components was plotted (Figure 5). There is a trend toward grouping by geographical location.

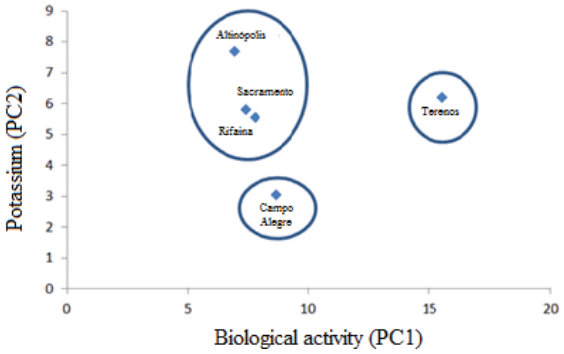

Figure 5. Principal components analysis (PCA) of five *Cochlospermum regium* populations.

This finding indicates that future agronomical evaluations of the fertilization and antimicrobial activity of *C. regium* species should prioritize investigation of potassium levels. Among numerous metabolic variations, low potassium levels in plants alter the production of Reactive Oxygen Species (ROS). In turn, higher ROS production affects ethylene and jasmonic acid synthesis (Shin, 2011). Ethylene plays an important role in the synthesis of phenolic compounds, even in roots (Lewis et al., 2011; Muday, Rahman, & Binder, 2012; Kramer et al., 2012). This role could justify the effect of low potassium levels on the antimicrobial action of *C. regium* roots, which contain biologically active phenolic compounds as the main constituents (Sólon et al., 2012).

Conclusion

Seasons of the year affect the concentration of antimicrobials in *Cochlospermum regium* roots. Fall/winter promote the highest antimicrobial activity, probably because the plant experiences hydric stress during these periods. Furthermore, adult plants cultivated in poor soils display higher antimicrobial action. Therefore, we concluded that phytotherapic medications should be made from whole roots collected during the fall or winter. Furthermore, our studies confirmed the efficiency of *C. regium* as a medicinal plant; the results showed antimicrobial activity in several types of environment and plant growth stages. Therefore, the use of this plant by the location population for medicinal purposes was validated.

Acknowledgements

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant# 2010/15168-6) for financial support as well as the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Cruz, C. D. (2006). *Programa Genes – Biometria* (p. 382). Viçosa, MG: UFV.

Çırak, C., Radušienė, J., Janulis, V., & Ivanauskas, L. (2007). Secondary metabolites in *Hypericum perfoliatum*: variation among plant parts and phenological stages. *Botanica Helvetica*, *117*(1), 29-36.

Ebrahimi, S. N., Hadian, J., Mirjalili, M. H., Sonboli, A., & Yousefzadi, M. (2008). Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. *Food Chemistry*, *110*(4), 927-931.

Faleiro, F. G., & de Farias Neto, A. L. (2008). *Savanas: desafios e estratégias para o equilíbrio entre sociedade, agronegócio e recursos naturais*. Planaltina, DF: Embrapa Cerrados.

Ferreira, D. F. (2011). SISVAR: a computer statistical analysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042.

Graybill, J. R., Burgess, D. S., & Hardin, T. C. (1997). Key issues drugs concerning fungistatic *versus* fungicidal. *European Journal of Clinical Microbiology & Infectious Diseases*, *16*(1), 42-50.

Honda, N. K., Brum, R. L., Hess, S. C., Cruz, A. B., & Moretto, E. (1997). Antibacterial activity of *Cochlospermum regium* essential oil. *Fitoterapia*, *68*(1) 79-79.

Inácio, M. C., Carmona, F., Paz, T. A., Furlan, M., Silva, F. A., Bertoni, B. W., França, S. C., & Pereira, A. M. S. (2013). Screening test for antibiotics in medicinal plants (STAMP): using powdered plant materials instead of extracts. *American Journal of Plant Science*, *4*(12), 2340-2350.

Instituto de Metereologia [INMET]. (2013). *Brazilian Meteorology Institute*. Retrieved September 5, 2013 from http://www.inmet.gov.br.

Kramer, M., Bufler, G., Ulrich, D., Leitenberger, M., Conrad, J., Carle, R., & Kammerer, D. R. (2012). Effect of ethylene and 1-methylcyclopropene on bitter compounds in carrots (*Daucus carota* L.). *Postharvest Biology and Technology*, *73*(1), 28-36.

Lewis, D. R., Ramirez, M. V., Miller, N. D., Vallabhaneni, P., Ray, W. K., Helm, R. F., Winkel, B. S. J., & Muday, G. K. (2011). Auxin and ethylene induce flavonol accumulation through distinct transcriptional networks. *Plant Physiology*, *156*(1), 144-164.

Lima, D. P., Castro, M. S. A., Mello, J. C. P., Siqueira, J. M., & Kassab, N. M. A. (1996). A flavanone glycoside from *Cochlospermum regium*. *Fitoterapia*, *66*(6), 545-546.

Moreira, D. L., & Guarim-Neto, G. (2009). Usos múltiplos de plantas do Cerrado: um estudo etnobotânico na comunidade sítio Pindura, Rosário Oeste, Mato Grosso, Brasil. *Polibotánica*, *27*, 159-190.

Muday, G. K., Rahman, A., & Binder, B. M. (2012). Auxin and ethylene: collaborators or competitors? *Trends in Plant Science*, *17*(4), 181-195.

Nunes, G. P., Silva, M. F., & Rezende, U. M. (2003). Medicinal plants from herb sellers operating in downtown Campo Grande, Mato Grosso do Sul, Brazil. *Revista Brasileira de Farmacognosia*, *13*(2), 83-92.

Oliveira, C. C., Siqueira, J. M., Souza, K. C. B., & Rezende, U. M. (1996). Antibacterial activity of rhizomes from *Cochlospermum regium*: preliminary results. *Fitoterapia*, *67*(2), 176-177.

Pavarini, D. P., Pavarini, S. P., Niehues, M., & Lopes, N. P. (2012). Exogenous influences on plant secondary metabolite levels. *Animal Feed Science and Technology*, *176*(1-4), 5-16.

Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, *6*(11), 1720-1731.

Sartorelli, P., Marquioreto, A. D., Amaral-Baroli, A., Lima, M. E. L., & Moreno, P. R. H. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. *Phytotherapy Research*, *21*(3), 231-233.

Selmar, D., & Kleinwächter, M. (2013). Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops and Products*, *42*(1), 558-266.

Shin, R. (2011). Transcriptional regulatory components responding to macronutrient limitation. *Journal of Plant Biology*, *54*(5), 286-293.

Sólon, S., Brandão, L. F. G., & Siqueira, J. M. (2009). Genus *Cochlospermum* Kunth with emphasis on ethnobotanic, pharmacological, toxicological, and chemical aspects of the *Cochlospermum regium* (Mart. Et. Schr.) Pilger. *Revista Eletrônica de Farmácia*, *6*(3), 1-22.

Sólon, S., Carollo, C. A., Brandão, L. F. G., Macedo, C. S., Klein, A., Dias-Junior, C. A., & Siqueira, C. M. (2012). Phenolic derivatives and other chemical compounds from *Cochlospermum regium*. *Química Nova*, *35*(6), 1169-1172.

Souza, C. D., & Felfili, J. M. (2006). The utilization of medicinal plants in the region of Alto Paraíso of Goiás, GO, Brazil. *Acta Botanica Brasilica*, *20*(1), 135-142.

Szakiel, A., Pączkowski, C., & Henry, M. (2011). Influence of environmental abiotic factors on the content of saponins in plants. *Phytochemistry Reviews*, *10*(4), 471-491.

Zachos, F. E., & Habel, J. C. (2011). *Biodiversity Hotspots: distribution and protection of conservation priority areas.* Heidelberg, BW: Springer.

Received on January 14, 2016.
Accepted on March 31, 2016

Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v38i4.30567