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Influence of plant part, season of collection and content of the main active constituent, on the antifungal properties of *Polygonum acuminatum* Kunth.

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ABSTRACT

Ethnopharmacological relevance: Polygonum acuminatum Kunth. (Polygonaceae) is used to heal infected wounds and as antifungal in the traditional Argentinean medicine.
Aim of the study: The present investigation was carried out to evaluate the antifungal properties of aerial parts of *Polygonum acuminatum*, in order to give support to its ethnopharmacological use as antifungal agent and to isolate the compound(s) responsible for the antifungal properties. The influence of the plant part used, the season of the year and a study of the correlation of the antifungal activity with the content of the main active constituent were investigated too, with the aim of contributing to determine the most suitable plant extract and season of the year for achieving the best antifungal properties for *Polygonum acuminatum* traditional use.
Materials and methods: For the antifungal evaluation, the microbroth dilution assay recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) was used against a panel of human opportunistic and pathogenic fungi. Bioassay-guided fractionation allowed us to isolate the compounds responsible for the antifungal activity. GC–MS was used to quantify the main component in the different extracts. For the statistical analysis, ANOVA test for analyses of variance followed by the Tukey test of Multiple Comparisons were used. The correlations between content of the antifungal compound and antifungal activity, were calculated with the Spearman Correlation Coefficient.
Results: Aerial parts (A) of *Polygonum acuminatum* showed to possess antifungal properties against yeasts as well as dermatophytes but not against *Aspergillus* spp. From the most active extract (ADCM), polygodial, isopolygodial, drimenol and confertifolin were isolated, possessing polygodial a broader spectrum of action and lower MICs than the rest of compounds. Among the different parts, leaves (L), stems (S) and fruits (F), that constitute the aerial parts of *Polygonum acuminatum*, (L) showed to possess the best activities, compared to (S) and (F).

The analysis of the content of polygodial in the LHex, LDCM, LEtOAC, LMeOH extracts collected in Summer, Autumn, Winter and Spring showed that LDCM of all seasons possessed higher percentages of this sesquiterpene than the rest of extracts. Among the LDCM of different seasons, that of Autumn was the most concentrated in polygodial. The correlation between content of polygodial with antifungal behavior of the different extracts, showed that LDCM of Autumn contains the highest content of polygodial and concomitantly the lowest MICs.
Conclusion: The ethnopharmacological use of *Polygonum acuminatum* aerial parts in the Argentinean traditional medicine for ailments related to fungal infections is supported by the results obtained in this investigation. From the obtained results, LDCM of Autumn, possessing the highest content of polygodial and the lowest MICs, appeared to be the most suitable extract for being used as antifungal in the traditional medicine. Nevertheless, if some other plant collection of another season different from Autumn is available, a LDCM extract would be the better option, because it contains a higher amount of polygodial compared to LHex, LEtOAc or LMeOH and therefore, a better antifungal activity can be expected.

1. Introduction

Since the early 1980s, fungal infections have emerged as major causes of morbi-mortality, mainly among immunocompromised patients. The majority of deaths were associated with species of

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Candida, *Aspergillus* and *Cryptococcus* (Pfaller and Diekema, 2007). Instead, dermatophytes such as *Trichophyton* and *Microsporum* spp. produce superficial infections (tineas) which are usually not threatening but dramatically diminish the quality of life of human beings (Weitzman and Summerbell, 1995).

Although it appears to be an array of antifungal agents (polyenes, azoles, allylamines and the recent echinocandins) there are, in fact, few therapeutic options. Decreased susceptibilities of yeasts to the currently available antifungal agents (Hsueh et al., 2005) added to the increase in the number of reported cases of resistance (White et al., 1998, 2002), have led to a general consensus that new efforts for detecting novel antifungal entities remain a priority.

In this context, the study of plants with history of ethnopharmacological use for ailments related to fungal infections, can serve two goals: validation of the use of traditional medicines and finding new leads (Verpoorte, 2000).

The genus *Polygonum* (Polygonaceae), which comprises about 300 species (Wang et al., 2005) is distributed worldwide in temperate climates. It is well known for producing a variety of plant secondary metabolites such as phenylpropanoids (Murai et al., 2001; Takasaki et al., 2001), acetophenones (Yoshizaki et al., 1987), chalcones (López et al., 2006), coumarins (Sun and Sneden, 1999), flavonoids (López et al., 2006), lignans (Kim et al., 1994), naphtoquinones (Kimura et al., 1983), anthraquinones (Qi et al., 2005), sesquiterpenoids (Datta et al., 2000; Derita et al., 2008; Jacobsson and Muddathir, 1992), triterpenoids (Duwiejua et al., 1999), stilbenoids (Xiao et al., 2000) and tannins (Wang et al., 2005; Silva et al., 1999).

This genus is represented in Argentina by 21 species, which are divided in five sections: *Echinocaulon*, *Amblygonum*, *Persicaria*, *Tiniaria* and *Polygonum* (Cialdella, 1989; Gattuso, 2000).

In the course of our current study on species of *Persicaria* section (López et al., 2006; Derita et al., 2008), we report here the antifungal properties of different extracts of aerial parts of *Polygonum acuminatum*, which is used in the Argentinean traditional medicine to heal infected wounds and for other ailments related to fungal infections (Petenatti et al., 1997; Del Vitto et al., 1998; Hieronymus, 1882). In addition, the bioassay-guided fractionation of the most active extract is also reported along with the compounds responsible for the antifungal activity. A study of the seasonal variation of the most active compound in the different extracts of leaves, stems and fruits and a correlation with the antifungal activity of each extract are reported too. These data contribute to determine the most suitable plant extract and season of the year for achieving the best antifungal properties for *Polygonum acuminatum* traditional use.

2. Materials and methods

2.1. Plant material

Aerial parts of *Polygonum acuminatum* Kunth. (*Polygonaceae*) were collected at *Puerto Gaboto*, Santa Fe province, in the arm "Boca del ternero" of Coronda river. The navigation of about 1 km through this arm of the river, led us to a left shore zone where wild *Polygonum acuminatum* plants grow. We delimitated an area of 100 m^2 with 20 wild plants inside. Aerial parts of these plants were collected in four different months of the year: March (Autumn), June (Winter), September (Spring) and December (Summer) of 2006. The plants were identified by Prof. Susana Gattuso from the National University of Rosario (UNR) and a *voucher specimen* of each collection was deposited at the Herbarium of the Vegetal Biology Area of UNR [Suipacha 531-(2000)-Rosario, Argentina, UNR 1672, 1673, 1674 and 1675 respectively].

2.2. Preparation of extracts

Air-dried aerial parts (A, 700 g) of plants collected in Autumn, were powdered in a Fristzch Pulverisette-15 mill (Germany). The material was successively macerated (3 × 24 h each) with petroleum ether (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH), with mechanical stirring using a Heidolph RZR 50 (Schwabach, Germany). After filtration and evaporation AHex (9.36 g), ADCM (14.74 g), AEtOAc (8.79 g) and AMeOH (19 g) extracts were obtained.

In turn, leaves (L), stems (S) and fruits (F) collected in the different seasons of the year were extracted with the same solvents used for aerial parts giving LHex, LDCM, LEtOAc, LMeOH, SHex, SDCM, SEtOAc, SMeOH, FHex, FDCM, FEtOAc, FMeOH extracts for each season.

2.3. Bioassay-guided fractionation of ADCM

ADCM (14.74 g) was chromatographed on Silica gel and eluted successively with Hex–EtOAc gradient (100% Hex to 100% EtOAc). After TLC comparison [Silica gel GF 254, Hex:EtOAc: (80:20) as the mobile phase; detection under UV light and spraying with sulfuric acid solution (10%)], fractions with similar TLC patterns were combined. The obtained 15 fractions were tested for antifungal activity. Repeated column chromatographies of fractions 5–8 led to the isolation of the sesquiterpenes polygodial (**1**) (1925 mg), isopolygodial (**2**) (70 mg), drimenol (**3**) (30 mg) and confertifolin (**4**) (35 mg). Pure compounds were identified by micromelting point, optical rotation and spectroscopic data including ^{1}H and ^{13}CNMR (Ying et al., 1995; Barnes and Loder, 1962; Guillerm et al., 1984) and were compared with authentic samples obtained previously in our laboratory (Castelli et al., 2005; Malheiros et al., 2005; Derita et al., 2008 for polygodial and isopolygodial) or with literature data (Rodríguez et al., 2005; Urban and Capon, 1996, for drimenol; Hueso-Rodríguez and Rodríguez, 1989; Appel et al., 1963, for confertifolin).

2.4. Quantification of polygodial in extracts from L, S and F collected in different seasons of the year

LHex, LDCM, LEtOAc, LMeOH, SHex, SDCM, SEtOAc, SMeOH, FHex, FDCM, FEtOAc, FMeOH extracts were submitted to GC–MS using a Turbo Mass PerkinElmer chromatograph, equipped with a fused silica gel column (SE-30 25 m × 0.22 mm ID) with He as a carrier gas, coupled to a mass selective detector, film 0.25 μm, ionization energy 70 eV with a temperature programme of 70–200 °C at 10 °C/min; total time 30 min. Polygodial was identified by comparison of its retention time (17.62 min) and the MS spectrum with an authentic sample obtained from our previous works (Castelli et al., 2005; Malheiros et al., 2005; Derita et al., 2008). The linearity of the detector response was verified using a series of two-fold diluted DCM solutions of polygodial. The relationship between peak areas (detector responses) and amount of polygodial was linear over 1000–31.2 μg/mL.

2.5. Statistical analysis

All data were expressed as the mean ± standard deviation (S.D.). Residuals were tested for normality and homoscedasticity using the Shapiro Wilk (Shapiro and Wilk, 1965) and the Bartlett tests (Snedecor and Cochran, 1989). Comparison of the content of polygodial was performed using multiple comparison analyses of variance (ANOVA) (Lindman, 1974) followed by the Tukey test of Multiple Comparisons (Miller, 1981). Means in the same column with different letters (a–c) are significantly different ($P < 0.05$) between

groups. The correlations between content of polygodial and antifungal activity, were calculated with the Spearman Correlation Coefficient *r* (Moore, 2006).

2.6. Antifungal assays

2.6.1. Microorganisms and media

For the antifungal evaluation, strains from the American Type Culture Collection (ATCC, Rockville, MD, USA) and Centro de Referencia en Micología, CEREMIC [C, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531 (2000)-Rosario, Argentina] were used: *Candida albicans* ATCC 10231, *Candida tropicalis* C 131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATTC 26934, *Aspergillus niger* ATCC 9029, *Trichophyton rubrum* C 110, *Trichophyton mentagrophytes* ATCC 9972 and *Microsporum gypseum* C 115. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C, maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Inocula of cell or spore suspensions were obtained and quantified following reported procedures (NCCLS, 2002; Wright et al., 1983).

2.6.2. Bioautography

Chromatograms were placed in sterile petri dishes with covers (Rahalison et al., 1991). Sabouraud growth medium with 0.6% agar and 0.02% phenol red (1 mL/cm^2) containing an inoculum of *Cryptococcus neoformans* ATTC 32264 of 1–5 × 10^5 cells final concentration, quantified according to reported procedures (Wright et al., 1983) was distributed over TLC plates containing the samples. After solidification of the medium, the TLC plates were incubated overnight at 28 °C. Subsequently, bioautograms were sprayed with an aqueous solution (1 mg/mL) of methylthiazolyltetrazolium chloride (MTT) and incubated for another 2 h at 28 °C. Dark yellow inhibition zones appeared against a dark brown background (Saxena et al., 1995).

2.6.3. Microbroth dilution assays

Minimum Inhibitory Concentration (MIC) of each extract or compound was determined by using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standard Institute [CLSI, formerly National Committee for Clinical and Laboratory Standards (NCCLS)] for yeasts (M27-A2) and for filamentous fungi (M38A) (NCCLS, 2002). MIC values were determined in Sabouraud broth (Britania, Buenos Aires) culture medium. Microtiter trays were incubated at 35 °C for yeasts and hialohyphomycetes and at 28–30 °C for dermatophyte strains in a moist, dark chamber. MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi.

For the assay, stock solutions of extracts and pure compounds (100 μL) were two-fold diluted with the culture medium. A volume of 100 μL of inoculum suspension [adjusted to 1–5 × 10^4 cells/spores with colony forming units (CFU/mL)] was added to each well with the exception of the sterility control where sterile water was added to the well instead. Concentrations from 500 to 0.98 μg/mL and a final DMSO concentration ≤2% were obtained in the different wells. Ketoconazole (Sigma Chem. Co., St. Louis, MO), Terbinafine (Novartis) and Amphotericin B (Sigma) were used as positive controls. After determining the MIC, an aliquot of 5 μL sample was withdrawn from each clear well of the microtiter tray and plated onto a 150 mm Sabouraud agar plate. Inoculated plates were incubated at 30 °C, and Minimum Fungicide Concentration (MFC) was recorded after 48 h. The MFC was defined as the lowest concentration of each compound that resulted in total inhibition of visible growth in these plates. Extracts or compounds with MICs and MFCs ≤500 μg/mL were considered active.

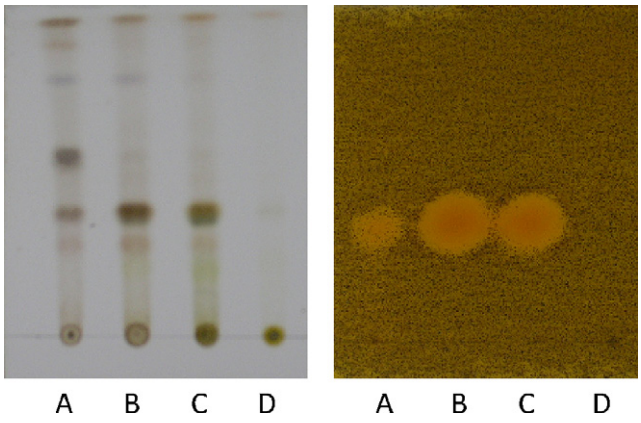


Fig. 1. Left: TLC of aerial parts extracts of *Polygonum acuminatum* Kunth. developed with Hex:EtOAc (80:20). Lanes A: AHex (petrol ether extract); B: ADCM (dichloromethane extract); C: AEtOAc (ethyl acetate extract); D: AMeOH (methanol extract). Right: Bioautography of the TLC showed in the left, with *Cryptococcus neoformans* ATCC 32264.

3. Results and discussion

3.1. Antifungal activity of Polygonum acuminatum aerial parts. Bioassay-guided fractionation of the most active extract

AHex, ADCM, AEtOAc and AMeOH extracts of aerial parts of a sample of *Polygonum acuminatum* collected in Autumn (March 2006) were first evaluated for antifungal properties with bioautography using *Cryptococcus neoformans* ATCC 32264. This bioassay showed that AHex, ADCM, AEtOAc (Fig. 1, lanes A–C) but not AMeOH (lane D) possess antifungal activity, giving us the additional information that the activity of all active extracts would be due to the same band which can be clearly observed in Fig. 1, at right.

The four extracts were then evaluated with the microbroth dilution assay following the guidelines of CLSI, up to 500 μg/mL against a panel of opportunistic and pathogenic fungi. Results showed (Table 1) that all yeasts and dermatophytes, but not *Aspergillus* spp., were sensitive to *Polygonum acuminatum* extracts.

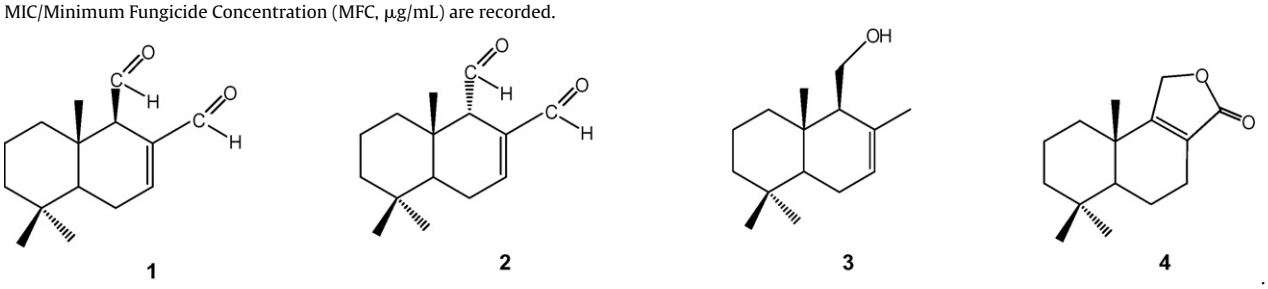
ADCM displayed the broadest spectrum of action, inhibiting six out of the nine fungi tested with MICs between 31.25 and 500 μg/mL; in turn, both the AHex and AEtOAc extracts inhibited 4/9 fungi, with MICs 125–500 μg/mL. AMeOH extract was inactive (MIC > 500 μg/mL) in all fungi tested. Based on these results (Table 1, rows 1–4), the most active ADCM extract was submitted to bioactive-guided fractionation in order to isolate the compound(s) responsible for the activity.

The fractionation of the ADCM extract gave 15 fractions which were evaluated against the most sensitive fungi. Results showed (Fig. 2) that the activity was concentrated mainly on fractions 5–8 for all fungi tested.

Repeated chromatographies of fractions 5–8 led to the isolation of polygodial (**1**) as the main component and isopolygodial (**2**), drimenol (**3**) and confertifolin (**4**) in lower percentages (see structures in Table 1), which have been previously isolated from another natural sources (Jansen and de Groot, 2004; Muñoz-Concha et al., 2007).

Results on the antifungal activity of **1**–**4** against the same panel used for extracts, are shown in Table 1. The best activities were displayed by polygodial **1**, which possesses a broad spectrum of action and high activity against *Candida albicans* (MIC = 3.90 μg/mL), *Saccharomyces cerevisiae* (MIC = 15.62 μg/mL), *Cryptococcus neoformans* and *Trichophyton* spp. (MICs = 7.81 μg/mL) and moderate

Table 1
Minimum Inhibitory Concentration (MIC, μg/mL) of petrol ether (AHex), dichloromethane (ADCM), ethyl acetate (AEtOAc) and methanol (AMeOH) extracts of aerial parts of *Polygonum acuminatum* collected in Autumn and of the isolated pure compounds against a panel of human opportunistic and pathogenic fungi. For pure compounds MIC/Minimum Fungicide Concentration (MFC, μg/mL) are recorded.



Extract	C a	S c	Cn	A fu	A fl	A n	M g	T r	T m
AHex	*i*	*i*	500	*i*	*i*	*i*	125	125	125
ADCM	125	500	62.5	*i*	*i*	*i*	125	31.25	31.25
AEtOAc	*i*	*i*	250	*i*	*i*	*i*	125	125	125
AMeOH	*i*	*i*	*i*	*i*	*i*	*i*	*i*	*i*	*i*
Compounds isolated from ADCM									
Polygodial (**1**)	3.90/7.81	15.62/31.25	7.81/7.81	*i*	*i*	*i*	62.5/125	7.81/31.25	7.81/15.62
Isopolygodial (**2**)	*i*	*i*	62.5/125	*i*	*i*	*i*	62.5/125	62.5/125	62.5/125
Drimenol (**3**)	*i*	*i*	125/>250	*i*	*i*	*i*	62.5/125	62.5/125	62.5/125
Confertifolin (**4**)	*i*	*i*	*i*	*i*	*i*	*i*	125/250	62.5/125	62.5/125
Standard drugs									
Amp.	1	0.50	0.25	0.50	0.50	0.50	0.12	0.07	0.07
Ket.	0.50	0.50	0.25	0.12	0.50	0.25	0.04	0.02	0.02
Terb.	–	–	–	–	–	–	0.04	0.01	0.04

C a, *Candida albicans* ATCC 10231; S c, *Saccharomyces cerevisiae* ATCC 9763; Cn, *Cryptococcus neoformans* ATCC 32264; A fu, *Aspergillus fumigatus* ATCC 26934; A fl, *Aspergillus flavus* ATCC 9170; A n: *Aspergillus niger* ATCC 9029; M g, *Microsporum gypseum* C 115; T r, *Trichophyton rubrum* C113; T m, *Trichophyton mentagrophytes* ATCC 9972. Amp., Amphotericin B; Ket., Ketoconazole; Terb., Terbinafine. *i*: >500 μg/mL

activity against *Microsporum gypseum* (MIC = 62.5 μg/mL). Compounds **2** and **3**, with a narrower spectrum of action, were inactive against *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus* spp. and moderately active against *Cryptococcus neoformans* and dermatophytes (MIC = 62.5 μg/mL). Compound **4** was moderately active only against dermatophytes. The four compounds possess fungicide rather than fungistatic activities (see MFC values in Table 1).

The activity displayed by isopolygodial adds a new evidence to the finding of Sterner (Anke and Sterner, 1991) who reported that, in contrast with previous suggestions (Taniguchi et al., 1984), **2** possesses antifungal activity, although lower than **1**.

Polygodial, the drimane type dialdehyde sesquiterpene was first isolated in 1962 from *Polygonum hydropiper* L. (Polygonaceae) (Barnes and Loder, 1962) and furtherly from species of *Warburgia* (Canellaceae) (Kubo et al., 1976), *Pseudowintera* (Winteraceae) (Perry et al., 1996), *Tasmania* (Dragar et al., 1998) and *Drimys* (Muñoz-Concha et al., 2007). Regarding the species of Polygonaceae, **1** was also isolated from *Polygonum punctatum* (Almeida Alves et al., 2001) and recently it was detected in *Polygonum acuminatum* and *Polygonum persicaria* (Derita et al., 2008), all spp. belonging to *Persicaria* section. The first report of its capacity for inhibiting fungi was provided by McCallion et al. (1982) and subsequently by Anke and Sterner (1991) and by Lee et al. (1999).

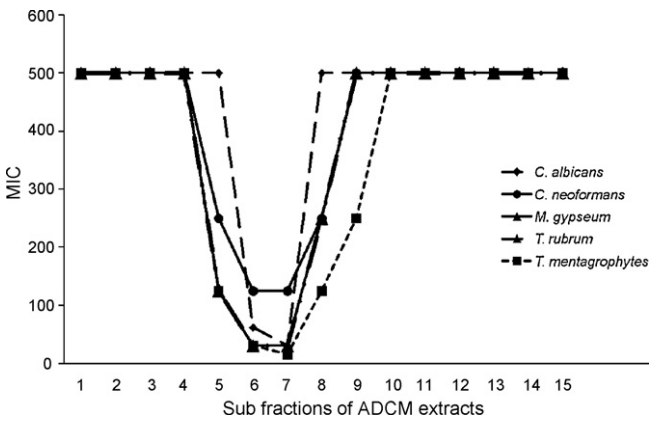


Fig. 2. Minimum Inhibitory Concentration of the fifteen fractions obtained from DCM extract of *Polygonum acuminatum* aerial parts, against a panel of human pathogenic and opportunistic fungal strains.

Taking into account that Kubo and other researchers reported that polygodial also acts as insect antifeedant agent (Kubo et al., 1976; Mori and Watanabe, 1986; Powell et al., 1993) it is possible that, according to the season of the year, the content of polygodial could vary in parallel with the presence or absence of insects (Koptur, 1985). Therefore, this variation could result in a modification of the antifungal properties of *Polygonum acuminatum*, being this datum important for the properly use of this plant in the traditional medicine.

So, we decided to study the content of polygodial in the different plant parts that constitute the "aerial parts" of *Polygonum acuminatum* in the different seasons of one year long. In addition, for each plant part, the antifungal activity of each extract was correlated with the content of **1** in order to determine the suitable plant part, the better extract and the most appropriate time of collection for obtaining the strongest antifungal activity.

3.2. Variation of the percentages of polygodial in extracts of different aerial parts of the plants collected in the four seasons of the year

The concentrations of polygodial (g/100 g of dry vegetal material) in Hex, DCM, EtOAc and MeOH extracts of leaves (L), stems (S) and fruits (F) collected in Summer, Autumn, Winter and Spring were determined by GC–MS according to Section 2.

Results showed that there was a significative variation in the total % of polygodial in the different aerial parts (ANOVA test,

Table 2
Percentage of polygodial (g/100 g of dry vegetal material) in petrol ether (Hex), dichloromethane (DCM), ethyl acetate (AcOEt) and methanol (MeOH) extracts of leaves (L), stems (S) and fruits (F) from *Polygonum acuminatum* collected in Summer, Autumn, Winter and Spring.

	Extract	Summer	Autumn	Winter	Spring
Leaves	LHex	0.20 ± 0.02	0.08 ± 0.00	0.18 ± 0.00	0.15 ± 0.01
	LDCM	0.26 ± 0.01	0.75 ± 0.02	0.49 ± 0.01	0.21 ± 0.01
	LAcOEt	0.18 ± 0.00	0.16 ± 0.01	0.17 ± 0.00	0.14 ± 0.02
	LMeOH	0	0	0	0
	Total in each season	0.64 ± 0.03	0.99 ± 0.03	0.84 ± 0.01	0.50 ± 0.04
Stems	SHex	0	0	0.08 ± 0.01	0.01 ± 0.00
	SDCM	0	0	0	0.01 ± 0.00
	SAcOEt	0	0	0	0.01 ± 0.00
	SMeOH	0	0	0	0
	Total in each season	0	0	0.08 ± 0.01	0.03 ± 0.00
Fruits	FHex	0	0.05 ± 0.01	0.08 ± 0.01	–
	FDCM	0	0.30 ± 0.01	0.14 ± 0.01	–
	FAcOEt	0	0.11 ± 0.01	0	–
	FMeOH	0	0	0	–
	Total in each season	0	0.46 ± 0.03	0.22 ± 0.02	–

Table 3
Results of the Tukey Test of Multiple Comparisons. The content of polygodial (g/100 g dry vegetal material) in petrol ether, dichloromethane and ethyl acetate extracts from *Polygonum acuminatum* leaves (LHex, LDCM, LEtOAc), collected in Summer, Autumn, Winter and Spring of one year long, are compared. Different letters indicate significant differences among values.

Extract	Summer Media	Autumn Media	Winter Media	Spring Media
LHex	0.20 ± 0.02 a	0.08 ± 0.00 a	0.18 ± 0.00 a	0.15 ± 0.01 a
LDCM	0.26 ± 0.01 b	0.75 ± 0.02 c	0.49 ± 0.01 b	0.21 ± 0.01 b
LEtOAc	0.18 ± 0.00 a	0.16 ± 0.01 b	0.17 ± 0.00 a	0.14 ± 0.02 a

Table 4
Minimum Inhibitory Concentration (MIC) of dichloromethane extracts of leaves (LDCM) of *Polygonum acuminatum*, collected in the four seasons of one year long.

	Content of polygodial (g/100 g of dry leaves)	MICs (μg/mL)			
		Ca	*Cn*	*Mg*	*Tm*
Summer	0.26 ± 0.01	500	500	500	125
Autumn	0.75 ± 0.02	125	62.5	125	31.25
Winter	0.49 ± 0.01	250	125	250	62.5
Spring	0.21 ± 0.01	500	500	500	250

$P < 0.001$) possessing L the highest total content of polygodial in all seasons [0.64%, 0.99%, 0.84% and 0.50% in Summer, Autumn, Winter and Spring respectively (Table 2)].

To determine which L extract significantly differ one each other, we applied the Tukey test of Multiple Comparisons, that show that LDCM significantly differs in the content of polygodial from LHex and LEtOAc in all seasons (Table 3).

The variation of content of polygodial in L collected in different seasons showed a similar profile (Fig. 3) for each extract: lower in LHex and LEtOAc than in LDCM. Within LDCM extracts, that from Autumn was two or more times richer in polygodial than the obtained in Winter, Summer and Spring respectively.

In order to determine if the content of polygodial was statistically correlated to the antifungal behavior of each extract, we determined the antifungal activity of the LDCM obtained in different seasons against four representative species of fungi (*Candida albicans*, *Cryptococcus neoformans*, *Microsporum gypseum* and *Trichophyton mentagrophytes*).

Results showed (Table 4) that the lowest MICs were obtained with the LDCM collected in Autumn, which coincides with the highest content of polygodial.

These results were corroborated by using the Spearman Correlation Coefficient ρ, which is a quantitative measure of the association between two variables and can adopt ρ values between (−1) and (+1). Negative or positive values are indicative of negative or positive gradients of a straight line and the nearer (−1) or (+1) gradient value, the higher association between variables.

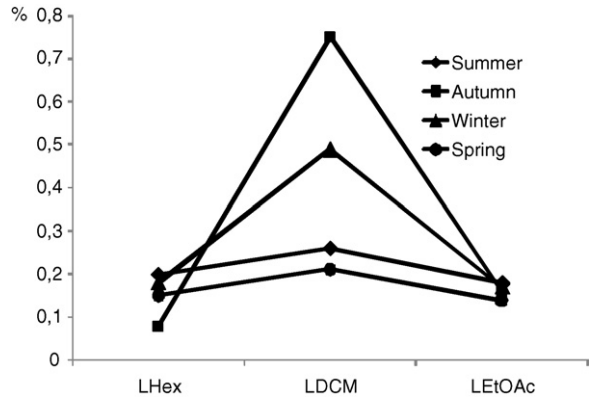


Fig. 3. % Polygodial (g/100 g dry vegetal material) in petrol ether, dichloromethane and ethyl acetate extracts from leaves (LHex, LDCM, LEtOAc), collected in different seasons of a year.

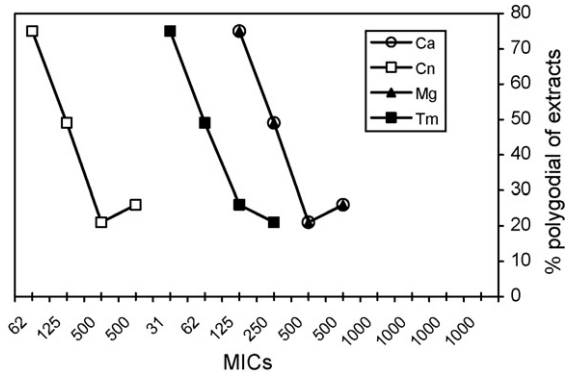


Fig. 4. Minimum Inhibitory Concentration of DCM extracts of leaves of *Polygonum acuminatum vs.* their content in polygodial expressed as $(g \times 10^{-2})/100$ g of dry vegetal material. Content of polygodial in leaves collected in Spring: 0.21%; in Summer: 0.26%; in Winter: 0.49%; in Autumn: 0.75%. Note: Lines for *Candida albicans* and *Microsporum gypseum* superimpose.

Fig. 4 shows the lines obtained by correlating the % of polygodial of LDCM collected in different seasons with MICs of extracts against *Candida albicans*, *Cryptococcus neoformans*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. Results showed that the gradient of lines are negative, indicating that extracts containing a higher concentration of polygodial display better antifungal activities.

From the above results, it is clear that LDCM of Autumn, possessing the highest content of polygodial and the lowest MICs, appeared as the most suitable extract for being used in the traditional medicine for ailments related to fungal infections. Nevertheless, if a collection of another season different from Autumn is available, a LDCM extract would be the better option, because it contains a higher amount of polygodial compared to LHex, LEtOAc or LMeOH and therefore a better antifungal activity can be expected.

In addition, considering that in a recent paper we suggested to delimitate the section *Persicaria* to those spp. of the *Polygonum* genus containing polygodial (Derita et al., 2008), this paper adds the interesting data that, whether our suggestion is accepted, it could be expected that all species belonging to the *Persicaria* section will possess antifungal properties due to the presence of the sesquiterpene dialdehyde polygodial.

4. Conclusions

Polygonum acuminatum aerial parts, used in the Argentinean traditional medicine for ailments related to fungal infections, possess antifungal properties against yeasts and dermatophytes, these results supporting their ethnopharmacological use.

From the most active extract (ADCM), polygodial was isolated as the main compound responsible for the activity, along with isopolygodial, drimenol and confertifolin, which displayed lower antifungal activities and possessed narrower spectra of action.

Among the different aerial plant parts, leaves (L) possessed the best activities, compared to stems (S) and fruits (F).

The analysis of the content of polygodial (**1**) in the LHex, LDCM, LEtOAC and LMeOH extracts collected in Summer, Autumn, Winter and Spring showed that in all seasons, LDCM possessed higher percentages of **1** than the rest of extracts. Among the LDCM extracts of different seasons, that of Autumn was the most concentrated in polygodial.

In addition, the Spearman Correlation Coefficient showed that a higher percentage of polygodial in extracts, positively correlates with a higher antifungal activity represented by lower MICs.

Based on the above results, we can conclude that LDCM would be the most suitable extract for being used as antifungal in traditional medicine, since it contains higher percentage of polygodial and lower MICs than other parts of the plant and other extracts of diverse polarity all year long. Regarding the season for collection, LDCM extract prepared from plants collected in Autumn would be the best choice because it showed to possess a higher content of polygodial than LDCM from plants collected in Summer, Winter or Spring and therefore, a better antifungal activity can be expected.

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