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Survey of the *In Vitro* Antifungal Activity of *Paeonia broteri* Extracts

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Abstract

Objective: Some species of *Paeonia* are widely studied as they are used in traditional Chinese medicine as an anti-inflammatory, antioxidant, antitumor, immune-system-modulation and antiseptic. There is still a lack of knowledge about other temperate Eurasia species. Such is the case of *P. broteri*, an Iberian endemism whose preliminary studies have the purpose to evaluate antifungal properties of their roots, leaves and fruits extracts.

Methods: The microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of *P. broteri* extracts against 13 selected fungi and yeasts: *Aspergillus niger*, *Candida albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans*, *Geotrichum* sp., *Rhodotorula rubra*, *Saccharomyces cerevisiae* and *Trichosporon cutaneum*. *P. broteri* extracts where obtained by sequential extraction of increasing polarity (n-hexane, CH_2Cl_2, EtOAc, MeOH and H_2O) and their phytochemical profile was screened.

Results: *P. broteri* roots and leaves extracts were the most active, in particular against *Geotrichum* sp, *T. cutaneum* and *A. niger*. The chemical profile of these extracts, flavonoids, phenols and terpenoids, may be responsible for their antifungal activity.

Conclusion: Studies with *P. broteri* extracts indicate that they may have antifungal potential.

Keywords: *Paeonia broteri*; Antifungal activity; Phytochemical screening; Microdilution method

Introduction

Chemotaxonomic studies placed the genus *Paeonia* L. in the monogeneric family Paeoniaceae Raf. [1-4]. Their 35 species are distributed in 3 sections: Moutan, Oneapia, and Paeonia, all living in the temperate areas of North hemisphere [5]. Some *Paeonia* species have a long tradition of use in Chinese medicine, among them *Paeonia lactiflora* Pallas (Syn: *P. albiflora Pallas*, *P. edulis Salisb*, *P. officinalis* Thunb) [6-9]. Phytochemical studies on these and on a few other Paeonia taxa, have revealed more than 262 compounds, belonging to different chemical groups: monoterpenoid glucosides, triterpenoids, steroids, stilbenoids, flavonoids, tannins and phenols [10,7]. Several biological and pharmacological activities, such as an antiseptic, anti-inflammatory, antioxidant, antitumor, immune-system-modulation, cardiovascular-system and central-nervous-system, have been assigned to those chemicals [7,11-13]. In Portugal *P. broteri Boiss*. & Reut. (Syn: *P. broteroi*, *P. lusitanica*) is endemic and can be found in understory of oak populations [14]. To our knowledge few local western species have been studied so far. Our objective was to evaluate the antifungal properties of *P. broteri* extracts, obtained from roots, leaves and fruits, against selected pathogenic fungi.

Materials and Methods

Plant material

Samples of tuberous roots, leaves and fruits of *P. broteri* were collected from native populations, in a mountainous area of Southwestern Portugal (38º 8'N-8º 33'W). Plant material was identified, and voucher specimens were deposited in the Lisbon Botanical Garden Herbarium (LISU 221345).

Plant extracts

P. broteri samples were dried in the dark, at room temperature and ground into powder separately. About 100g of each powder

was extracted sequentially, with increasing polarity solvents, [n-hexane (n-hex), dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt), methanol (MeOH) and water (H_2O)], for 24h, at room temperature with occasional shaking. The obtained extracts were filtered, evaporated under reduced pressure and stored at 4ºC, until use, with the exception of the aqueous extract which has been freeze-dried.

Phytochemical screening

Each extract was dissolved in proper solvents, applied on silica gel thin layer chromatography plates and de¬veloped with appropriate mixtures of eluents. The plates, containing an application of each extract, were revealed with spray specific reagents for the presence of each main class of metabolites NEU for flavonoids, FBS for phenols, anisaldehyde / sulfuric acid for terpenoids and saponins, and Dragendorff for alkaloids [15]. Results were displayed semi-quantitative in a range between absent (-), slight (+), moderate (++) and strongly present (+++).

Fungi strains

The selected fungi included 13 filamentous fungi and yeasts: *Aspergillus niger* (ATCC 16888), Candida albicans (ATCC 90028), *C. dubliniensis* (FFUL 21), *C. glabrata* (FFUL 12B), *C. guilliermondii* (FFUL 1403), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 90018), *C. tropicalis* (ATCC 750), *Cryptococcus neoformans* (FFUL 948), *Geotrichum* sp. (ATCC 96884), *Rhodotorula rubra* (FFUL 190), *Saccharomyces cerevisiae* (FFUL 1997) and *Trichosporon cutaneum* (FFUL 18H).

Minimal inhibitory concentration

The microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of each of the *P. broteri* extracts against the selected fungi. The MIC is the lowest concentration that inhibits the development of a microorganism. The different extracts were tested in a concentration range between 500 - 7.5μg/mL and the culture medium used was the Sabouraud liquid. The microplates were incubated at 37°C for 24h, with the exception of *A. niger*, which required 48h of incubation period. All the assays were repeated at least in three independent experiments. Positive values were considered when MIC<100μg/mL [16] and verified by observing the wells turbidity.

Results

Phytochemical screening

The main classes of metabolites in each extract are shown in Table 1. The samples corresponding to the three plant structures do not have alkaloids, but their phytochemical profile shows some differences regarding the presence of flavonoids, phenols, terpenoids.

Table 1: Phytochemical screening of *P. broteri* roots, leaves and fruits extracts.

Samples	Extracts	Flavonoids	Phenols	Terpenoids	Alkaloids
Roots	n-hex	++	+++	+++	-
	CH_2Cl_2	-	-	++	-
	AcOEt	+	++	++	-
	MeOH	+	++	+	-
	H_2O	+++	++	+	-
Leaves	n-hex	+	++	++	-
	CH_2Cl_2	++	-	+	-
	AcOEt	+	+	+	-
	MeOH	++	+	+	-
	H_2O	++	++	-	-
Fruits	n-hex	-	+	++	-
	CH_2Cl_2	-	+	++	-
	AcOEt	+++	+	++	-
	MeOH	++	++	+	-
	H_2O	+	++	-	-

Antifungal activity

Table 2 shows the obtained MIC values of the *P. broteri* extracts against *A. niger*, *C. albicans*, *C. dubliniensis*, *Geotrichum* sp., *R. rubra*, and *T. cutaneum*. No interesting results were obtained against *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. neoformans* and *S. cerevisiae*. Considering the root samples, the n-hex extract strongly inhibited (MIC=7.5μg/mL) the growth of *A. niger* and of *G. candidum*; the AcOEt extract showed moderate inhibition (MIC=30μg/mL), against *C. albicans* and *C. dubliniensis* the MeOH extract moderately inhibited (MIC=30μg/mL) the

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growth of *Geotrichum* sp. and slightly inhibited (MIC=62.5µg/mL) the growth of *T. cutaneum* the H_2O extract strongly inhibited (MIC=7.5µg/mL) the growth of *A. niger* and of *Geotrichum* sp. and moderately inhibited (MIC=30µg/mL) the growth of *T. cutaneum*.

The results obtained with the leaves samples indicate that the n-hex extract moderately inhibited (MIC=30µg/mL) the growth of *A. niger* and strongly inhibited (MIC=7.5µg/mL) the growth of *Geotrichum* sp, the AcOEt extract showed a slight inhibition (MIC=62.5µg/mL) of growth of *Geotrichum* sp. and of *T. cutaneum* the MeOH extract had a slight inhibitory activity (MIC=62.5µg/mL) on *R. rubra* the H_2O extract inhibited strongly to moderately (MIC=15µg/mL) the growth of *A. niger*, strongly (MIC=7.5µg/mL) of *Geotrichum* sp. and moderately (MIC=30µg/ml) of *R. rubra* and of *T. cutaneum*. The activity of *P. broteri* fruit samples show that the n-hex extract moderately inhibited (MIC=30µg/mL) the growth of *T. cutaneum* the H_2O extract had a slight inhibition (MIC=62.5µg/mL) over *A. niger*, a moderate inhibition (MIC=30µg/mL) over *T. cutaneum* and a strong inhibition over *Geotrichum* sp. (MIC=7,5 µg/mL). Table 2 also shows that CH_2Cl_2 extracts had no inhibition at all against the tested fungi.

Table 2: Minimum inhibitory concentration (MIC µg/mL) of the *P. broteri* extracts against selected fungi. MIC values >100 µg/mL were considered as negative (-).

Samples	Extracts	*Aspergillus niger*	*Candida albicans*	*Candida dubliniensis*	*Geotrichum* sp	*Rhodotorula rubra*	*Trichosporon cutaneum*
Roots	n-hex	7,5	-	-	7,5	-	-
	CH_2Cl_2	-	-	-	-	-	-
	AcOEt	-	30	30	-	-	-
	MeOH	-	-	-	30	-	62,5
	H_2O	7,5	-	-	7,5	-	62,5
Leaves	n-hex	30	-	-	7,5	-	-
	CH_2Cl_2	-	-	-	-	-	-
	AcOEt	-	-	-	62,5	-	62,5
	MeOH	-	-	-	62,5	62,5	-
	H_2O	15	-	-	7,5	30	30
Fruits	n-hex	-	-	-	-	-	30
	CH_2Cl_2	-	-	-	-	-	-
	AcOEt	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	H_2O	62,5	-	-	7,5	-	30

Discussion

The phytochemical screening performed on *P. broteri* roots, leaves and fruits extracts showed some semi-quantitative differences regarding the presence of flavonoids, phenols, terpenoids and verified as well the absence of alkaloids. The MIC evaluations carried out with those extracts revealed antifungal activity against some of the tested fungi. In descending order, the most sensitive fungi were *Geotrichum* sp., *T. cutaneum*, *A. niger*, *R. rubra*, *C. albicans*, *C. dubliniensis* (Table 2). No antifungal activity was noticed against *C. tropicalis*.

Lai et al. [17] performed a phytochemical screening on crude organic extracts of leaves and fruits of *P. broteri* and evaluated their antimicrobial activity against standard and resistant strains of Gram-positive, Gram-negative bacteria, Mycobacterium smegmatis and *C. albican*. The same authors found that the most polar extracts strongly inhibited Staphylococcus aureus standard, VRSA and MRSA strains but no inhibition against *C. albicans* was noticed [17]. Picerno et al. [18] working with root polar extracts of *P. rockii* found a moderate inhibition (MIC=30µg/mL) against *C. albicans*. These same authors attributed the antifungal activity to the chemical composition of the mentioned extract, rich in phenolic compounds, gallic acid derivatives and paeoniflorina, a monoterpene glycoside [18]. The last compound seems to be ubiquitous in all the species examined of the Paeoniaceae family and it is considered as a chemotaxonomic marker [1,19]. Papandreou et al. [20] isolated volatile constituents from roots of 3 Paeonia species and detected their antimicrobial activity against pathogenic bacteria and against *C. albicans*, *C. tropicalis* and *C. glabrata*. *P. broteri* root n-hex extract revealed activity against *A. niger* and *Geotrichum* sp. and only the AcOEt root extract showed activity against *C. albicans* and *C. dubliniensis* (Table 2).

Conclusion

The phytochemistry of *P. broteri* was beyond the scope of our study but considering our results with those of the other authors,

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it is suggested that the biological activity of *P. broteri* extracts can be attributed to the synergistic effect of terpenoids, flavonoids and phenolics. The compounds involved and their mechanism of action are unknown and need to be elucidated. The activity of *P. broteri* extracts indicates that they may have potential as antifungal and as a source of new models of bioactive molecules, but further investigations are needed.

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Conflict of interests

The authors have no conflicts of interest to declare. The authors are entirely responsible for the paper's content and writing.

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