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Screening of antifungal activity of Rumex L. species

Badanie skryningowe aktywności przeciwgrzybiczej *Rumex* L.

INTRODUCTION

Nowadays, an important medical problem is searching for new antifungal drugs due to the increased incidence of fungal infections, including those caused by fungi resistant to available drugs. Moreover, about 200 fungal species may be etiologic agents of human diseases, while fungal infections are already present at about 10-20% of the population in the world [18].

The *Rumex* (dock) genus comprises of 25 species growing in Poland. Plant species of this genus have been well known for their use in traditional medicine all over the world since ancient times due to therapeutic efficacy and various biological activities [1]. Preparations from these plants, especially due to their anti-inflammatory and antimicrobial activities, are useful in skin infections [3, 9, 10, 14, 15, 20]. Antifungal properties of some *Rumex* L. species, *i.e. Rumex acetosa* L., *Rumex crispus* L. and *Rumex obtusifolius* L. growing in China, were reported for the first time in 1981 [8]. Some later reports suggest that only acetone extracts from roots were active against fungi, especially against yeasts belonging to *Candida albicans* [21].

The objective of the present study was to screen antifungal activity *in vitro* of the ethanol extracts obtained from fruits, leaves and roots of the six species of the *Rumex – R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L. growing in Poland, against the reference strains of fungi, representing yeasts, dermatophytes or moulds.

MATERIAL AND METHODS

P l a n t m a t e r i a l. Fruits, leaves and roots of six species of *Rumex* L.: *R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L. were used in this study. The plant material was collected in the natural places in the vicinity of Lublin in June 2006. It was dried for 15 days at room temperature in the absence of light in a well-ventilated place and then stored at room temperature in air-tight glass jar protected from light. The voucher specimens were deposited in Department of Pharmaceutical Botany, Medical

University in Lublin, Poland (voucher specimens no. RF.01.06 – RF.06.06, RL.01.06 – RL.06.06 and RR.01.06 – RR.06.06).

Preparation of plant extracts. The cut up (0.5 cm pieces), air-dried and finally powdered plant material (5 g) was extracted three times with 50 ml 95% aqueous ethanol and once with a solution of 25 ml 95% aqueous ethanol and 25 ml distilled water (15 minutes of each extraction) in supersonic water bath at room temperature. The combined extracts were concentrated under reduced pressure at 40ºC and the residue was stored in a dark place to completely evaporate the solvent. Then the dry, powered residues were placed in the Eppendorf's tubes, weighed and taken to further antimicrobial investigations. The rest of the extract was kept under refrigerated conditions.

Dry ethanol extracts from the fruits were dissolved under aseptic conditions in the DMSO (dimethyl sulfoxide) at a concentration of 50 mg/mL (stock solution).

Chemicals and reagent. Fluconazole was obtained from Sigma Aldrich (Germany). Ethanol was obtained from POCH Gliwice (Poland).

Test microorganisms. The microorganism panel used in the study consisted of 5 reference strains of fungi from American Type Culture Collection (ATCC) - yeasts (*Candida albicans* ATCC 2091, *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019), dermatophytes (*Trichophyton mentagrophytes* ATCC 9533) and moulds (*Aspergillus niger* ATCC 16404). The microbial suspensions of each microorganism was adjusted to 0.5 McFarland standard - 150 x 10^6 CFU (colony forming units)/mL in sterile saline (0.85% NaCl).

Antifungal activity screening. The determination of the antifungal activity of the ethanol extracts from leaves, fruits and roots of *Rumex* species was made *in vitro* with the use of the agar dilution method. Mueller-Hinton medium (Biocorp) supplemented with 2% glucose and buffered at pH 5.6, containing two-fold dilutions of the tested extracts (at final concentration 500, 250, 125, 62.5 and 31.25 μg/mL) was used. All stock solutions of the tested extracts were dissolved in dimethyl sulfoxide (DMSO). The final concentration of the DMSO in the medium had no influence on the growth of the tested fungi.

Microbial suspensions (0.025 mL) were put onto Petri dishes with 20 mL medium with or without the tested extracts. The incubation was carried out at 30˚C for 48 h for *Candida* spp. and for 3 to 5 days for *A. niger* ATCC 16404 and *T. menthagrophytes* ATCC 9533, depending on the growth in the control medium. The activity was expressed by visual assessment as the percentage of the reference fungi strains growth inhibition compared with the control growth observed on the medium without the extracts added. MIC (minimal inhibitory concentration), defined as the lowest concentration of the extracts required for 80% inhibition of the visible growth of the tested microorganisms, was also determined. Fluconazole was used as control antifungal agent against *Candida* spp.

RESULTS

Antifungal activity of the ethanol extracts from fruits, leaves and roots of the six species of the *Rumex* - *R. acetosa*, *R. acetosella*, *R. confertus*, *R. crispus*, *R. hydrolapathum* and *R. obtusifolius* was compared, using agar dilution method, allowing for MIC determination. According to our results (Table 1), among the tested ethanol extracts from the above species of the *Rumex* the most active against fugal species studied were the extracts obtained from fruits; the extracts from leaves were much less active, while the extracts from roots had no activity.

Table 1. Antifungal activity of ethanol extracts from fruits, leaves and roots of six species of *Rumex* determined by agar dilution method (percentage of growth inhibition)

Plant species	Organ	Concentration (µg/mL)	Fungi				
			Ca 2091	Ca 10231	Cp 22019	Tm 9533	An 16404
Rumex acetosa	fruits	250	40	40	40	50	0
		500	80	80	80	60	10
	leaves	250	0	0	0	0	0
		500	20	0	0	0	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0
Rumex acetosella	fruits	250	10	30	20	60	30
		500	80	80	90	70	50
	leaves	250	20	10	0	0	0
		500	30	0	0	0	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0
Rumex confertus	fruits	250	90	90	90	80	0
		500	100	100	100	100	10
	leaves	250	30	30	30	0	0
		500	40	40	40	0	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0
Rumex crispus	fruits	250	80	90	90	80	10
		500	100	100	100	100	20
	leaves	250	10	10	10	30	0
		500	20	10	20	40	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0
Rumex. hydrolapathum	fruits	250	90	90	90	80	10
		500	100	100	100	100	20
	leaves	250	10	10	20	30	0
		500	20	20	30	40	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0
Rumex. obtusifolius	fruits	250	90	90	70	80	10
		500	100	100	100	100	30
	leaves	250	10	10	10	10	10
		500	30	40	30	30	0
	roots	250	0	0	0	0	0
		500	0	0	0	0	0

Abbreviations: Ca 2091 - *Candida albicans* ATCC 2091, Ca 10231 - *Candida albicans* ATCC 10231, Cp 22019 - *Candida parapsilosis* ATCC 22019, Tm 9533 - *Trichophyton menthagrophytes* ATCC 9533, An 16404 - *Aspergillus niger* ATCC 16404

The order of growth inhibitory activity of fruit extracts at concentration of 250 μg/mL in case of the tested yeast species belonging to *Candida* spp. was as following: *R. confertus* = *R. hydrolapathum* (av. about 90%) > *R. crispus* (av. about 87%) > *R. obtusifolius* (av. about 83%) > *R. acetosa* (av. about 40%) > *R. acetosella* (av. about 20% inhibition of the growth). The order of growth inhibitory activity of fruit extracts at concentration of 500 μg/mL in case of this group of fungi was as following: *R. confertus* = *R. hydrolapathum* = *R. crispus* = *R. obtusifolius* (av. about 100%) > *R. acetosella* (av. about 83%) > *R. acetosa* (av. about 80%). The MICs for *R. confertus* and *R. hydrolapathum* were 250 μg/mL, for *R. crispus* and *R. obtusifolius* were 250-500 μg/mL or for *R. acetosa* and *R. acetosella* were 500 μg/mL. Fluconazole inhibited the growth of the reference strain of *Candida* spp. with MIC = 62.5 -125 μg/mL.

Moreover, the extracts from fruits of *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* at concentration of 250 μg/mL inhibited the growth of *T. menthagrophytes* ATCC 9533 belonging to dermatophytes in about 80%, but from fruits of *R. acetosa* and *R. acetosella* at the same concentration in about 50-60%. The extracts from fruits of *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* at 500 μg/mL concentration inhibited the growth of this fungal species in about 100%, but from fruits of *R. acetosa* and *R. acetosella* at the same concentration in about 60-70%. The MICs for *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* were 250 μg/mL, while for *R. acetosa* and *R. acetosella* were > 500 μg/mL.

The majority of fruit extracts had no activity against *Aspergillus niger* ATCC 16404 (MIC > 500 μg/mL); only ethanol extract from fruits of *R. acetosella* at 500 μg/mL concentration inhibited the growth of this mould in about 50%.

DISCUSSION

Superficial mycoses are the most common infections in the world. Generally, these infections localized on the outer layers of skin, hair and nails as well as on mucous membranes can cause severe discomfort and disability, thereby potentially impacting quality of life. It is well-known that superficial mycoses localized on skin and mucous membranes are caused predominantly by dermatophytes from genus *Trichophyton* and yeasts from genus *Candida* [7, 12, 13]. Therapy of superficial mycoses is based mainly on use of antifungal drugs, especially azoles or terbinafine. It should be noted that the limited efficacy of azoles may be due to increasing resistance as a result of overuse of these antifungals. Recently, several herbal specimens of antifungal activity may be regarded as alternative or adjunctive therapeutic options.

According to literature data [4-6,11,16,19], several species of *Rumex* possess antimicrobial activity, but the spectrum of this activity depends on plants species, part of plant, methods of extraction or geographical region. We have found that the ethanol extracts from fruits of *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* have moderate activity against yeasts and dermatophytes. Inhibitory effect against *C. albicans* was also observed by Borchardt et al. (2009) with methanol extracts from the seeds of *R. cripsus* and *R. verticillatus* L. using disc diffusion method [2]. In contrast, according to Ulukanli et al. (2005), the methanol and acetone extracts of the aerial parts of *R. crispus* had no activity against *C. albicans* ATCC 10231, while the acetone extracts from roots demonstrated significant antifungal activity, as determined by well diffusion method [17]. Similar

results were presented by Yildirim et al. (2001); only acetone extracts from roots of *R. crispus* were found to possess inhibitory activity against *C. albicans*, while ether, ethanol and hot water extracts from leaves were inactive [21].

Antifungal activity of *Rumex* species may be due to the presence of anthraquinones: nepodin, emodin, chrysophanol and physcion [8]. However, further studies are necessary to determine the factors and the types of agents responsible for antifungal effects of *Rumex* species.

CONCLUSION

We have found that the ethanol extracts from fruits of *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* may be useful in treatment of superficial mycoses caused by yeasts and/or dermatophytes as alternative or adjunctive therapeutic options.

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SUMMARY

Antifungal activity of the ethanol extracts obtained from fruits, leaves and roots of six species of *Rumex* L. (*R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L.) growing in Poland against the reference strains yeasts, dermatophytes and moulds was assessed by agar dilution method allowing for determination of minimal inhibitory concentration (MIC). The extracts from fruits of *R. confertus*, *R. crispus*, *R. hydrolapathum* and *R. obtusifolius* were the most active, showing moderate activity against *Candida* spp. and *Trichophyton mentagrophytes* ATCC 9533 with MIC = 250-500 μg/mL; no activity against *Aspergillus niger* ATCC 16404 was found. The obtained data indicate the fruits from the above *Rumex* species may be regarded as alternative or adjunctive agents in treatment of superficial mycoses.

Keywords: Rumex L., ethanol extracts, fruits, leaves, roots, antifungal activity

STRESZCZENIE

Aktywność przeciwgrzybiczą ekstraktów etanolowych z owoców, liści i korzeni sześciu rosnących w Polsce gatunków *Rumex* L. (*R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L.) wobec szczepów referencyjnych drożdżaków, dermatofitów i grzybów pleśniowych oznaczano metodą rozcieńczeń badanych ekstraktów w podłożu agarowym w oparciu o wartość minimalnego działania hamującego (MIC - minimal inhibitory concentration). Wyciągi z owoców *R. confertus*, *R. crispus*, *R. hydrolapathum* i *R. obtusifolius* charakteryzowały się zróżnicowaną aktywnością wobec *Candida* spp. i *Trichophyton mentagrophytes* ATCC 9533 (MIC = 250-500 μg/mL); nie wykazano aktywności wobec *Aspergillus niger* ATCC 16404. Uzyskane dane wskazują, że wyciągi etanolowe z owoców *Rumex* L. mogą być stosowane jako alternatywne lub wspomagające substancje w leczeniu grzybic powierzchniowych.

Słowa kluczowe: Rumex L., ekstrakty etanolowe, owoce, liście, korzenie, aktywność przeciwgrzybicza