Polish J. of Environ. Stud. Vol. 18, No. 3 (2009), 377-382

Original Research

Fungistatic Properties of Glucosinolates – a Reconnaissance Study

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Received: 13 October 2008
Accepted: 26 February 2009

Abstract

The aim of our study was to investigate fungistatic properties of glucosinolates from seeds of four cruciferous plants against *Candida albicans*, obtained from different sections of the gastrointestinal and respiratory tracts.

The examined extracts were observed to exert different effects on fungi depending on the site of their isolation. The strongest antimycotic activity was reported for the extract obtained from seeds of broccoli – the extract appeared more effective than fluconazole. In contrast, upon the activity of white mustard seeds extract the size of the growth inhibition zone was similar to that yielded by fluconazole. Promising results of our experiments suggest the need to further investigate the field.

Keywords: glucosinolates, GLS degradation products, antifungal activity, *Candida albicans*

Introduction

It is commonly known that a vegetable-rich diet lowers the risk of some cancers in humans. In vegetables of the Cruciferae family, including cabbage, broccoli, radish, Brussels sprouts and cauliflower, anticarcinogenic activity is ascribed to be the products of degradation of sulfuric glycosides – glucosinolates (GLS). The products of enzymatic or nonenzymatic degradation of GLS are released during culinary processing of vegetables [1] or in the alimentary tract during digestion [2, 3]. These compounds can act as anticarcinogens by decreased carcinogen activation through inhibition of phase I enzymes, increased detoxification by induction of the phase II enzymes that affect the xenobiotic transformations, inhibition of tumor cell growth and stimulation of apoptosis [4, 5]. Unfortunately, under some conditions, indole compounds have been reported to demonstrate mutagenic and carcinogenic activity [6, 4].

A high contribution of vegetables of the family *Brassicaceae* in a diet has been demonstrated to substantially reduce the risk of tumor incidence, especially in the case of tumors of lungs, gastrointestinal tract, prostate and ovaries [7, 4]. Epidemiological surveys point to great significance of GLS hydrolysis products in preventing diseases of the circulatory system [8] as well as infections of stomach induced by *Helicobacter pylorii* [7]. The activity against microorganisms, confirmed also in phytopathological investigations against fungi, has been mainly attributed to isothiocyanates released from aliphatic and arylic GLS [9, 10]. Unlike vegetables, seeds are characterized by a remarkably higher content of both aliphatic and arylic GLS [1, 11-13]. These characteristics of seeds as well as their fungistatic properties suggest the usability of GLS extracts from cruciferous seeds in medicine against fungi potentially pathogenic to man, their opportunistic forms in particular.

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Material and Methods

Experimental Material

The material of the study were 24 isolates of *Candida albicans*, obtained from different sections of the gastrointestinal tract (oesophagus, stomach, colon, intestines) and the respiratory tract (nasal swab, sputum, pharyngeal swab, bronchoscopic material) of patients of the Endoscopy Laboratory of the Municipal Hospital and of the Independent Public Unit of Tuberculosis and Pulmonary Diseases in Olsztyn. The isolates were identified based on morphological traits (macrocultures on Sabouraud's medium, microcultures on Nickerson's agar) and biochemical properties (fermentation and assimilation of saccharides) using assay keys by: Lodder and Kreger-van Rij [14] and Kurtzman and Fell [15].

Preparation of Plant Material

GLS Analysis

Seeds of four cruciferous plants: broccoli (*Brassica oleracea* L. var. *italica*, hybrid cultivar Husky), small radish (*Raphanus sativus* L., cultivar Saxa POL), white cabbage (*Brassica oleracea* L. var. *capitata*, cultivar Sława z Gołebiewa) and white mustard (*Sinapsis alba*, cultivar Rota), as well as incubated homogenates, were analyzed for GLS contents with the method of HPLC acc. to the Official Journal of European Communities [16].

Preparation of Plant Extracts Containing GLS Degradation Products

Seeds (10g) were homogenized with deionized water (50ml) for 2 minutes using an Ultra Turrax homogenizer with T25 tip. To run GLS hydrolysis by native myrosinase, the homogenate was transferred to a water bath in tightly-closed vessels and gently shaken at a temperature of 30ºC. After 48 hours, 4 ml of suspension were collected. The suspension was filtrated and the resultant filtrate was added back to the incubated homogenate, whereas the precipitate was analyzed for the content of GLS according to Ciska et al. [11]. Non-hydrolyzed GLS were not detected in the sample examined. Products of GLS degradation were extracted from homogenates with 25 ml of dichloromethane. The extraction was carried out three times and the extracts obtained were combined and gently condensed to 25 ml in a rotary evaporator.

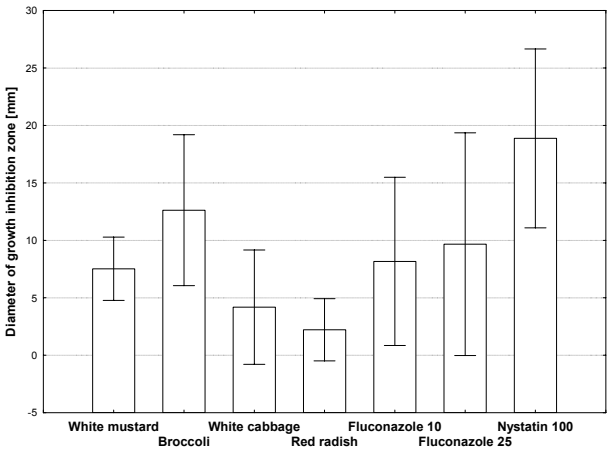

Fig. 1. Fungistatic properties of extracts obtained from seeds of cruciferous plants and drug-susceptibility of *C. albicans* isolates examined.

Evaluation of the Effect of an Extract from Seeds of Cruciferous Plants on *Candida albicans*

An analysis of fungistatic properties of glucosinolates degradation products was conducted with the diffusion-disk method. From the 24-h culture on liquid Sabouraud's medium, 1 ml was collected and suspended in 2 ml of sterile water. Next, 0.5 ml of the suspension obtained (10^6 cfu) were surface-inoculated onto solid Sabouraud's medium. Sterile disks blotting paper saturated with 100 µl of the extract were fixed onto the medium. After 24-h incubation at 37°C zones of fungi growth inhibition were measured around the disks. The experiment was conducted in triplicate.

Evaluation of Drug-Sensitivity of *C. albicans*

For comparative purposes, the effect of seed extracts on fungi growth was evaluated simultaneously with drug-sensitivity of the isolates examined to antimycotics commonly applied both in prophylaxis and treatment, i.e.: fluconazole (10 µg and 25 µg) and nystatin (100 u). Antimycogram was performed with the diffusion-disk method using disks by MAST DIAGNOSTICS.

Results

Contents of GLS in seeds used to prepare extracts of GLS degradation products were presented in Table 1. The type of identified compounds and their contents in particular seed species are consistent with results reported by [1, 12, 13]. The seeds displayed different contents of total GLS as well as their composition. Seeds of white mustard, small radish and broccoli were characterized by the presence of one major GLS. In seeds of broccoli and radish over 75% of total GLS were constituted by glucoraphanin and glucoraphenin belonging to aliphatic GLS. In seeds of white mustard the percentage of synalbin – representing arylic GLS – reached as much as 98%. In turn, seeds of cabbage were dominated by 3 aliphatic compounds: sinigrin – 38%, progoitrin – 30%, and glucoiberin – 13%.

The strongest antimycotic activity was reported for the extract obtained from seeds of broccoli. While applying that extract the mean zone of fungi growth inhibition accounted for 12.63 mm, whereas in the case of white

Table 1. GLS content (μmol/g) of seeds.

GLS	Red radish	White cabbage	Broccoli	White mustard
Glucoiberin	-	11.16	0.95	-
Progoitrin	-	25.04	0.34	-
Sinigrin	Tr*	31.87	0.44	3.29
Glucoraphanin	1.23	1.02	89.52	-
Glucoraphenin	75.27	-	-	-
Gluconapoleiferin	14.06	0.05	0.56	-
Glukonapina	-	2.71	0.05	-
Glucoiberverin	4.17	-	5.04	0.24
Glucoerucin	-	5.00	18.72	-
4-Methylthiobut-3-enyl GLS	0.72	-	-	-
Gluconasturtiin	-	0.68	-	-
Sinalbin	-	-	-	262.12
4-Hydroxyglucobrassicin	-	6.56	-	-
Glucobrassicin	0.28	0.41	1.24	0.18
4-Metoxyglucobrassicin	0.48	0.06	0.17	0.31
Neoglucobrassicin	0.35	-	0.19	-
Total	96.56	84.56	117.22	266.14

* Tr, trace < 0.05 μmol/g s.m.

mustard, cabbage and radish seed extracts it was 7.65, 4.21 and 2.25 mm, respectively (Fig. 1).

The extracts examined were observed to exert various effects on fungi depending on the site of their isolation (Fig. 2). The smallest growth inhibition zones upon the activity of the broccoli extract were reported for isolates originating from the stomach (6.5 mm), and the highest ones for those prepared from sputum (18.5 mm). Considerable differences in the size of the growth inhibition zone were also demonstrated upon the influence of the cabbage extract: from 0 mm for isolates from intestines to 8.64 mm for fungi from sputum. Fungi obtained from the respiratory tract did not react or reacted poorly to the extract of small radish seeds. Only in the case of sputum isolate did the mean growth inhibition zone reach 2.7 mm. In turn, all isolates displayed similar responses to the extract of white mustard, i.e. their growth inhibition zones fluctuated between 7.0 and 9.0 mm. An exception were fungi collected from intestines – the size of their growth inhibition zone equaled 3.5 mm (Table 2).

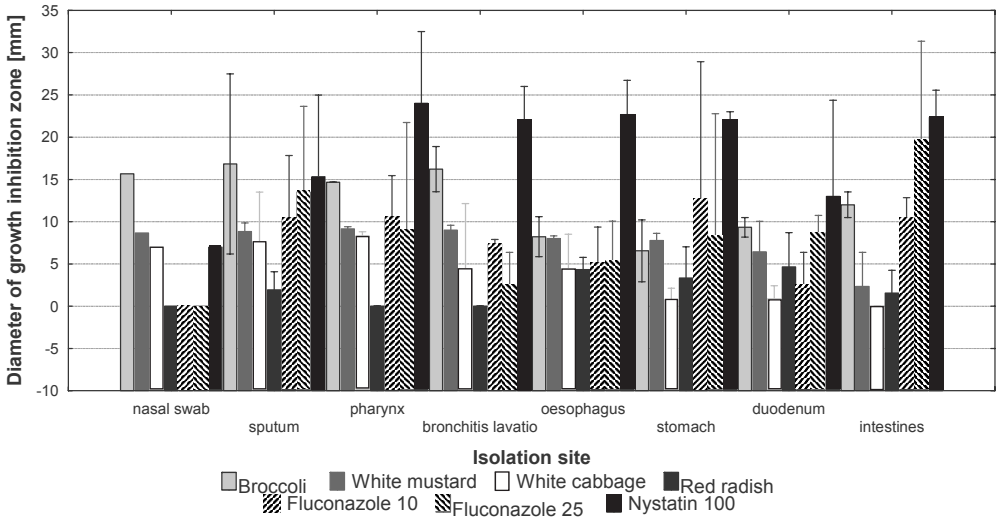

Fig. 2. Fungistatic properties of extracts obtained from seeds of cruciferous plants and drug-susceptibility of *C. albicans* isolates examined as affected by the site of isolation.

Table 2. Fungistatic properties of extracts obtained from cruciferous seeds and drug-susceptibility of *C. albicans* isolates examined.

Isolate number	Isolation site	Diameter of growth inhibition zone [mm]						
		Plant extracts				Antimycotics		
		Broccoli	White mustard	White cabbage	Red radish	Fluconazole 10	Fluconazole 25	Nystatin 100
62	nasal swab	15.7*	8.7	7.0	0	0	0	7.0
1	sputum	14.3	9.7	8.7	0	7.0	26.0	25.0
2	sputum	38.0	7.3	18.5	3.7	14.0	8.0	0
30	sputum	13.7	8.0	2.3	4.3	22.0	0	19.0
173	sputum	12.3	9.7	3.7	0	0	12.0	7.0
185	sputum	8.0	9.7	8.3	0	7.0	10.0	21.0
187	sputum	14.7	8.7	3.7	3.7	12.0	25.0	20.0
4	pharynx	14.7	9.0	8.0	0	7.0	0	18.0
79	pharynx	14.7	9.3	8.7	0	14.0	18.0	30.0
36	bronchitis lavatio	13.7	8.7	0	0	7.0	0	22.0
42	bronchitis lavatio	16.0	8.7	0	0	7.0	0	18.0
176	bronchitis lavatio	19.	9.7	13.3	0	8.0	7.0	26.0
64	oesophagus	5.7	8.3	5.3	5.3	7.0	0	19.0
66	oesophagus	8.7	8.0	0	5.0	0	7.0	22.0
67	oesophagus	10.3	7.7	8.0	2.7	8.0	9.0	27.0
86	stomach	8.7	8.7	0	2.7	31.0	25.0	23.0
97	stomach	2.3	7.0	2.3	0	0	0	21.0
123	stomach	8.7	7.7	0	7.3	7.0	0	22.0
92	duodenum	8.7	2.3	2.7	0	0	8.0	0
119	duodenum	8.7	8.0	0	7.0	0	11.0	18.0
124	duodenum	10.7	9.0	0	7.0	7.0	7.0	21.0
81	intestines	11.7	0	0	0	10.0	22.0	21.0
82	intestines	13.7	7.0	0	0	13.0	30.0	20.0
114	intestines	10.7	0	0	4.7	8.0	7.0	26.0

*in the case of plant extracts. the results provided are means of three replications

The isolates under study exhibited various susceptibility to commonly used antimycotics. Nystatin appeared more effective than fluconazole – the growth inhibition zone reached 18.87 mm. The action of both doses of fluconazole was much weaker than that of nystatin – mean zone below 10 mm (Fig. 1). The lowest susceptibility to nystatin was observed in the case of isolate originating from nasal swab – 7 mm, i.e. less than the value declared by the producer as a threshold between susceptibility and resistance. The greatest growth inhibition zones were obtained upon the action of nystatin for the isolates originating from pharynx, bronchoscopic material, oesophagus, stomach and intestines – over 20 mm (Fig. 2). Fluconazole applied at a dose of 10 µg appeared to be effective (diameter over 14 mm) in the case of 4 isolates from sputum, one from pharynx and one from stomach (Table 2). In turn, once applied at a dose of 25 µg it had the strongest inhibiting effect on fungi originating from intestines – the growth inhibition zone reached 19.67 (Fig. 2).

Discussion

The increasing percentage of potentially-pathogenic fungi as well as the rising number of cases of fungal infections pose a serious problem to contemporary medicine. Special attention is paid to the recently increasing resistance of yeast-like fungi to antimycotic drugs applied routinely in clinical practice. For several years investigations have been underway world wide into the search for new, alterna-

tive means of fungal infection treatment and prevention. World literature provides ample material on the use of biologically-active compounds of plant origin against the growth and development of potentially-pathogenic fungi. Fungistatic properties against anthropopathogens have been reported in experiments carried out with the use of extracted potato proteins [17]. Similar results were obtained while evaluating the fungistatic properties of essential oils [18, 19] that inhibited the growth of *C. albicans*, *Trichosporon rubrum* and *Geotrichum candidum*. Also, an extract of barberry was observed to exert the inhibiting effect on the growth of yeast-like fungi of the genus *Candida* (*C. albicans, C. glabrata, C. parapsilosis, C. luisitaniae, C. haemulonii*) [20]. Investigations carried out on fungi of the genus Candida and dermatophytes demonstrated that extracts of onion and garlic displayed antimycotic activity similar to that of ketoconazole [21]. Comparable effects were reported in a study with the use of a 33% grapefruit extract. It turned out to be effective in lower concentrations than ketoconazole and fluconazole [22]. The presented results of our assays partly correspond with literature data – the extract of broccoli seeds appeared more effective than fluconazole. In contrast, upon the activity of white mustard seeds extract the size of the growth inhibition zone was similar to that yielded by fluconazole.

Studies of the antimycotic properties of plants of the family Brassicaceae conducted by Sisti et al. [23] demonstrated inhibition of the growth and generation of germ tubes *C. albicans* by fresh cauliflower juice (*Brassica oleracea* var. *botrytis*). Similar results were obtained in our experiments for the extract of broccoli seeds. The low inhibiting effect of extracts from seeds of small radish and cabbage might be due to both lower content of total GLS and different GLS composition in those seed species. The diversified composition of GLS could, probably, also elucidate the substantially lower activity of the extract obtained from white mustard seeds, in which the content of GLS was twice as much as in broccoli seeds. The activity of the examined extracts could result from their mutual amount relations and individual properties of the examined isolates.

Differences in the response of particular strains to the extract are likely to result from individual ecophysiological properties of fungi, especially of the enzymatic apparatus, which reacts to every change in macro- or microenvironment [24]. Isolates originating from various ontospheres are adapted for conditions occurring in a given ontocenosis. The access of oxygen, pH, nutrients, defense mechanisms of a microorganism, as well as the composition of saprophytic microflora, differ in both systems (gastrointestinal and respiratory one), and even between their particular sections. Colonizing their own habitats, fungi have activated their own enzymatic pathways. Thus, they display diversified susceptibility to mycostatics [25, 26], including GLS.

The application of compounds of plant origin is an alternative to the use of synthetic antimycotic agents in the case of infections, and it provides an opportunity for their protective application, for instance during long-standing antimycotic antibiotic therapy. Promising results of our experiments suggest the need for further investigations in that field. The fungistatic properties of GLS degradation products point to the possibility of applying cruciferous seeds as a pharmaceutical raw material for the acquisition of antimycotic compounds. They afford the opportunity to elaborate easily degradable drugs. What is more, the incorporation of those compounds to diets of risk-group persons could contribute to a reduction in the number of mycotic infections.

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