Tropical Journal of Pharmaceutical Research March 2015; 14 (3): 449-454
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i3.13

Original Research Article

Evaluation of Glucosidase Inhibitory and Cytotoxic Potential of Five Selected Edible and Medicinal Ferns

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Received: 1 October 2014 *Revised accepted: 9 February 2015*

Abstract

Purpose: *To evaluate the glucosidase inhibitory and cytotoxic activities of five selected edible and medicinal ferns, namely, Blechnum orientale, Davallia denticulata, Diplazium esculentum, Nephrolepis biserrata, and Pteris vittata.*
Methods: *The aqueous extracts of the five ferns were prepared by water extraction at 90 ºC for 1 h. Antiglucosidase assay was used to determine the effect of each extract on yeast alpha-glucosidase activity in vitro. Cytotoxicity was evaluated using methylthiazol tetrazolium assay on chronic myelogenous leukaemia cell line (K562). The phenolic, hydroxycinnamic acid, flavonoid and proanthocyanidin contents of the extracts were also determined.*
Results: *The α-glucosidase inhibitory activity of D. esculentum (half maximal effective concentration, EC_{50} = 6.85 µg/ml) was considerably stronger than that of myricetin (EC_{50} = 53.21 µg/ml). B. orientale, D. esculentum, N. biserrata, and P. vittata were cytotoxic to K562 cells. P. vittata had the strongest cytotoxicity, although it was less potent than 5-fluorouracil. D. denticulata had the highest phenolic, hydroxycinnamic acid and flavonoid contents of all the extracts while B. orientale had the highest proanthocyanidin content.*
Conclusion: *Among the five ferns evaluated, D. esculentum is a potential source of an antidiabetic agent and is recommended for further investigation in this regard. All the fern extracts, except D. denticulata, exhibited dose-dependent cytotoxicity against K562 cells.*

Keywords: *Medicinal fern, α-Glucosidase inhibition, Cytotoxicity, Blechnum orientale, Davallia denticulata, Diplazium esculentum, Nephrolepis biserrata, Pteris vittata*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Worldwide, many fern species are used as traditional remedies for human diseases and also consumed as vegetables. Ferns are rich in natural products with therapeutically-relevant bioactivities, including anti-cancer, antioxidant, and anti-inflammatory activities [1]. Hence, ferns are promising bioresources for the discovery of bioactive compounds that can be exploited for the development of nutraceutical, cosmetic, and pharmaceutical products [1,2].

This study focused on five medicinal and edible ferns, namely *Blechnum orientale* L. (Blechnaceae), *Davallia denticulata* (Burm.) Mett. (Davalliaceae), *Diplazium esculentum* (Retz.) Sw. (Athyriaceae), *Nephrolepis biserrata* (Sw.)

Schott (Nephrolepidaceae), and *Pteris vittata* L. (Pteridaceae). *B. orientale, D. esculentum* and *N. biserrata* are edible ferns [3-6]. Besides being consumed as vegetable, *B. orientale* is used as a folk remedy for conditions including boils, headache, and flu [4]. *D. esculentum* is used to treat fever, dermatitis, and measles in ethnomedicine [7]. *N. biserrata* is used to treat malaria [8] as well as boils, abscesses, and blisters [9]. There is no documentation on the use of *P. vittata* as a vegetable. In traditional medicine, the fern is used for the remedy of abdominal pain, diarrhoea, and flu [4]. Information on the medicinal and food uses of *D. denticulata* is scarce. However, related species such as *D. fejeensis* [10], *D. mariesii* [4], and *D. formosana* [11] are well-known as traditional remedies for bone injuries and other disorders.

Phytochemicals with cytotoxic and antiglucosidase activities can be used in the development of chemopreventive and anti-diabetic therapies [12,13]. Alpha (α)-glucosidase is one of the key therapeutic targets in the management of type 2 diabetes mellitus. Hence, antiglucosidase natural products may be incorporated into nutraceuticals and functional food, and in turn, used in the management of diabetes [1,2,14]. The antiglucosidase activities of the five ferns have not been previously investigated. Likewise, nothing is known about the cytotoxic or anticancer potential of *N. biserrata* and *P. vittata*. Information on the anticancer effects of the other three ferns is also limited.

The health-promoting and therapeutic effects of the various classes of phenolic compounds derived from ferns and other plants have been established [1,14]. However, information on the polyphenol, hydroxycinnamic acid, flavonoid, and proanthocyanidin contents of the five selected ferns is limited. Hence, to fill in gaps of knowledge about the bioactivities of these ferns and to identify promising fern species for isolation of active compounds in future, this study had two objectives: first, to assess the antiglucosidase and cytotoxic activities of the aqueous extracts of the five ferns; second, to determine whether such bioactivities can be attributed to the phenolic, hydroxycinnamic acid, flavonoid, and proanthocyanidin contents of the five selected ferns.

EXPERIMENTAL

Plant materials

Healthy specimens of *B. orientale, D. denticulata, D. esculentum, N. biserrata*, and *P. vittata*, were collected from the countryside of Bidor town, Malaysia, in February 2013. The ferns were authenticated by Professor Dr Hean-Chooi Ong, a botanist at the University of Malaya, Malaysia. Voucher specimens of *B. orientale, D. denticulata, D. esculentum, N. biserrata*, and *P. vittata* (numbered TTC01/2013(1), TTC01/2013(2), TTC01/2013(3), TTC01/2013(4), and TTC01/2013(5), respectively) were deposited at the Faculty of Science, Universiti Tunku Abdul Rahman, for future reference.

Preparation of aqueous extracts

The fern samples were oven-dried at 45 ^{o}C for 72 h and then pulverised to powder with a Waring blender. Aqueous extracts were prepared by suspending the powder in autoclaved, deionised water at a ratio of 1:20 (dry weight: volume). The mixture was heated in a 90 ^{o}C water bath for 1 h. The extract was vacuum-filtered and the resulting filtrate was centrifuged at 7830 rpm at 4 ^{o}C for 5 min. The supernatant obtained was freeze-dried to constant weight and extract yield was recorded. The freeze-dried extract was then redissolved in deionised water to prepare aliquots of 50 mg/ml, which were then stored at -20 ^{o}C until further use.

Determination of α-glucosidase inhibitory and cytotoxic activities

α-glucosidase inhibitory activity was assessed as previously described [15]. A reaction mixture containing 250 µL of 100 mM potassium phosphate buffer (pH 7.0), 150 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside, 50 µL of extract, and 150 µL of α-glucosidase (0.1 unit/mL in 10 mM potassium phosphate buffer, pH 7.0) was incubated at 37 °C for 30 min. The reaction was terminated by adding 600 µL of 200 mM Na_2CO_3. The absorbance of the reaction mixture was recorded at 400 nm. A blank was prepared for each measurement by substituting α-glucosidase with 10 mM potassium phosphate buffer. Antiglucosidase activity (Ag) was calculated according to Eq 1.

$$Ag\ (\%) = \{1- (As /Ac)\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where As is the absorbance of control reaction (without extract) and Ac is the absorbance in the presence of an extract. Myricetin was used as the positive control. The effectiveness of myricetin as an α-glucosidase inhibitor has been established [16]. Half maximal effective concentration (EC_{50}) value, defined as the concentration of extract or myricetin required to achieve 50 % antiglucosidase activity, was determined using linear regression analysis.

Cytotoxicity of the extracts against the human chronic myelogenous leukaemia cell line (K562) was assessed by using a methylthiazol tetrazolium (MTT) assay as previously described [17]. 5-Fluorouracil, an anticancer drug, was used as the positive control. EC_{50} value, defined as the concentration of extract or 5-fluorouracil required for achieving 50 % cytotoxic activities, was determined by using linear regression analysis.

Phytochemical contents

Total phenolic (TP) content of the extracts was determined by using a Folin-Ciocalteu colorimetric assay [18] and expressed as mg gallic acid equivalents (GAE) per g of extract. Total hydroxycinnamic acid (THC) content was determined by using Arnow's reagent [19] and expressed as mg caffeic acid equivalents (CAE) per g of extract. Total flavonoid (TF) content was determined by using an aluminium chloride colorimetric assay [20] and expressed as mg quercetin equivalents (QE) per gram of extract. Total proanthocyanidin (TPro) content was assessed by the acid-butanol assay [21] and expressed as mg leucocyanidin equivalents (LE) per gram of extract.

Statistical analysis

Experiments were carried out in triplicates. Data presented are mean ± standard error of the mean (SEM). Statistical analyses were performed using Statistical Analysis System (SAS) software (version 9.2). Data were analysed by one-way ANOVA test and means of significant differences were separated using Fisher's least significant difference (LSD) test or Student's t test at α = 0.05. Linear regression and correlation analyses were carried out using Microsoft Office Excel 2007.

RESULTS

The yield of the extracts was as follows: 18.1 % (*B. orientale*), 18.0 % (*D. denticulata*), 22.4 % (*D. esculentum*), 17.6 % (*N. biserrata*), and 19.3 % (*P. vittata*).

Concentration-dependent increases in α-glucosidase inhibitory activity was observed for all the extracts, except *D. denticulata* (Fig 1). EC_{50} values for antiglucosidase activity among the extracts, ranked in ascending order, were *D. esculentum* < *B. orientale* < *P. vittata* (Table 1). The EC_{50} value of *D. esculentum* extract was considerably lower than that of myricetin. The EC_{50} values of *B. orientale* and *P. vittata* extracts were 24 % and 64 % higher than that of myricetin, respectively. *D. denticulata* showed α - glucosidase activation activity; hence its EC_{50} value was not determined.

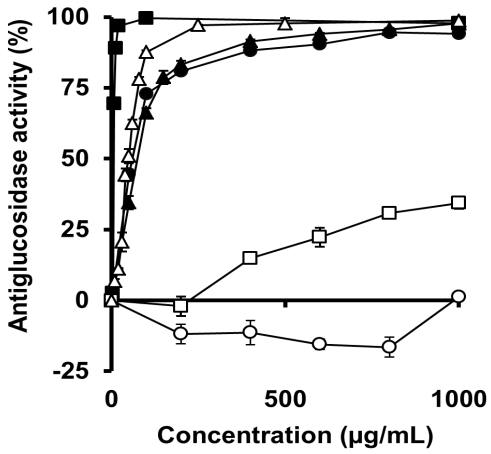

Fig 1: α-Glucosidase inhibitory activity of extracts of *B. orientale* (●), *D. denticulata* (○), *D. esculentum* (■), *N. biserrata* (□), and *P. vittata* (▲), compared with myricetin (Δ). Data points are mean ± SEM (*n* = 3)

Table 1: Antiglucosidase and cytotoxic activities of fern extracts (expressed as EC_{50}), compared with myricetin and 5-fluorouracil

Species	EC_{50} (µg/ml)	
	Antiglucosidase activity	Cytotoxic activity
B. orientale	65.78 ± 1.45 *	[a]
D. denticulata	ND	[b]
D. esculentum	6.85 ± 0.08 *	[a]
N. biserrata	[a]	[a]
P. vittata	87.00 ± 2.58 *	364.82 ± 15.94 *
Positive control	53.21 ± 0.91 (Myricetin)	212.86 ± 7.89 (5-fluorouracil)

Values are mean ± SEM (n = 3). Asterisks (*) denote values that are significantly different (p < 0.05) compared with positive control, as determined using Student's t test. ND = α-glucosidase inhibitory activity was undetectable. [a] EC_{50} was not calculated because maximum activity was below 50 %; [b] EC_{50} was not calculated due to lack of increasing trend in activity

All fern extracts, except *D. denticulata*, showed dose-dependent cytotoxicity toward K562 cells (Fig 2). The cytotoxic effect of *D. denticulata* extract decreased with increasing extract concentrations. The EC_{50} value for cytotoxic activity of *P. vittata* was 1.7-fold higher compared with 5-fluorouracil (Table 1).

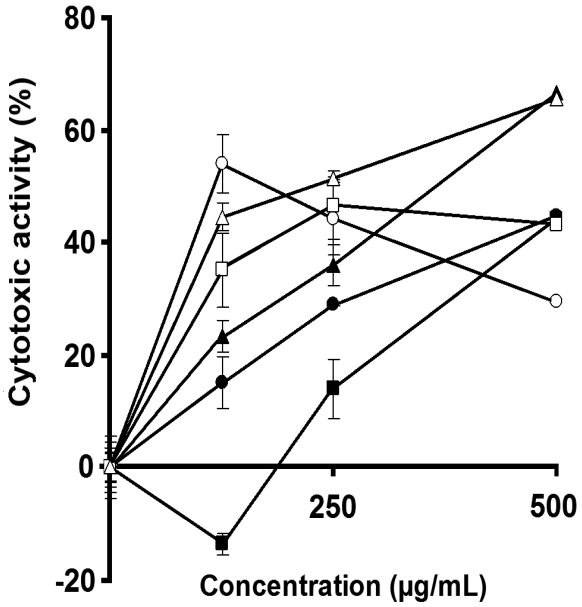

Fig 2: Cytotoxic activity of extracts of *B. orientale* (●), *D. denticulata* (○), *D. esculentum* (■), *N. biserrata* (□), and *P. vittata* (▲), compared with 5-fluorouracil (Δ). Data points are mean ± SEM (*n* = 3)

Overall, the five fern extracts investigated contained various levels of phytochemicals (Table 2). Phytochemical analysis revealed that *D. denticulata* extract had the TP, THC and TF contents, while *B. orientale* had the highest TPro among the five extracts. There was no significant, positive correlation between the phytochemical parameters and bioactivity with respect to the two parameters analysed in this study. *D. denticulata* extract was rich in TF (64 % by weight).

DISCUSSION

This study demonstrated for the first time the α-glucosidase inhibitory activity of *B. orientale, D. esculentum, N. biserrata*, and *P. vittata*. Importantly, despite being a crude extract, the edible fern *D. esculentum* was a more potent glucosidase inhibitor than myricetin. Our findings are relevant to the current interests in searching for natural antidiabetic agents and managing diabetes by dietary intervention [22,23]. The water-soluble and heat-stable nature of the α-glucosidase inhibitors in *D. esculentum* is evident in this study. Such α-glucosidase inhibitors could be readily extracted with water and their activity is likely to be preserved after cooking with heat.

In this study, we assessed the antiglucosidase activity of the extracts by using the yeast α-glucosidase. Yeast α-glucosidase is commercially available in pure form and is often used as a model for evaluating antiglucosidase potential of natural products [15,24,25]. Antiglucosidase plant extracts have been shown to significantly repress postprandial hyperglycemia in Streptozocin-induced diabetic mice [25]. Thus, although preliminary, our finding of the potent antiglucosidase activity of *D. esculentum* substantiates its use for treating diabetes in traditional medicine [26].

To the best of our knowledge, this report is the first account of the cytotoxicity of *P. vittata* and *N. biserrata* towards any cancer cell line. Our findings have also added valuable information to the currently limited knowledge on the cytotoxic potential of *B. orientale* and *D. esculentum*.

Table 2: Phytochemical contents of fern extracts

Species	Phytochemical content			
	***TP* (mg GAE/g)**	***THC* (mg CAE/g)**	***TF* (mg QE/g)**	***TPro* (mg LE/g)**
B. orientale	175.38 ± 9.58 [a]	150.33 ± 2.00 [a]	470.91 ± 7.87 [a]	30.47 ± 2.51 [a]
D. denticulata	212.64 ± 1.33 [b]	201.67 ± 2.65 [b]	639.09 ± 1.39 [b]	0.56 ± 0.10 [b]
D. esculentum	141.18 ± 10.51 [c]	86.33 ± 2.24 [c]	329.39 ± 2.98 [c]	0.62 ± 0.08 [b]
N. biserrata	59.37 ± 0.55 [d]	55.67 ± 0.36 [d]	184.85 ± 1.52 [d]	0.53 ± 0.08 [b]
P. vittata	70.59 ± 1.04 [d]	57.50 ± 0.63 [d]	155.76 ± 1.84 [e]	9.97 ± 0.55 [c]

Values are mean ± SEM (n = 3). Values in the same column that are followed by different superscript letters are significantly different (p < 0.05), as determined by using Fisher's LSD test. TP - total phenolics, THC - total hydroxycinnamic acids, TF - total flavonoids, TPro - total proanthocyanidins

Previously, organic and water fractions of methanol extract of *B. orientale* were found to be non-cytotoxic to K562 cells, although they were cytotoxic to human colonic adenocarcinoma cells (HT-29) and human colonic carcinoma cells (HCT-116) [27]. In this study, hot water extract of *B. orientale* was cytotoxic to K562 cells, although the level of cytotoxicity detected was not as high as that of *P. vittata*. Ethanolic extract of *D. esculentum* exhibit no notable cytotoxicity against breast, colon, and liver cancer cell lines [5]. Likewise, hydroethanol extract of *D. esculentum* was not cytotoxic to human cervical carcinoma (HeLa) cell line [28]. The present study demonstrated for the first time that *D. esculentum* was cytotoxic to K562 cells, although its effect is not as potent as that of *P. vittata*. In general, except for *D. denticulata*, the other four ferns investigated, especially *P. vittata*, are promising sources of water-soluble and heat-stable cytotoxic agents. Future research to isolate and identify cytotoxic constituents from *P. vittata* will be of great value to therapeutic agent development, especially that for leukaemia treatment. Moreover, the edible ferns *B. orientale, D. esculentum*, and *N. biserrata* may be of interest to future research aimed at discovering anticancer drugs of food origin or developing functional food with anticancer potential.

There were no correlations between bioactivity and phytochemical parameters in our study. Phenolic constituents of ferns and other plants are known to exhibit potent antiglucosidase and cytotoxic activities [1,29]. Hence, a possible explanation for our observations is that the five fern extracts may contain phenolic constituents that vary considerably in their efficacy or specific activity per unit mass as antiglucosidase or cytotoxic agents. Consequently, their bioactivities are not proportional to and cannot be readily predicted from their total phytochemical contents. Nevertheless, we cannot rule out the possibility that there may be classes of phytochemicals not analysed here which may have contributed to the bioactivities observed in the fern extracts. The nature of the active compounds responsible for the antiglucosidase activity of *D. esculentum* and the cytotoxicity of *P. vittata* can only be confirmed when the compounds are isolated from the ferns and structurally characterised.

Our study revealed that *D. denticulata* extract was rich in flavonoids (64 % by weight). Fern flavonoids are known to have diverse bioactivities [1]. Thus, such high abundance of flavonoids implies that *D. denticulata* may possess other bioactivities despite its lack of antiglucosidase and cytotoxic activities. There are no previous reports on the TP contents of *D. denticulata*. The observed higher TP content of *B. orientale* than for *P. vittata, N. biserrata* and *D. esculentum* are in agreement with the findings of an earlier work [30].

CONCLUSION

The antiglucosidase activity of *B. orientale, D. esculentum, N. biserrata*, and *P. vittata* are demonstrated here for the first time. Importantly, the aqueous extract of edible fern *D. esculentum* is a very potent α-glucosidase inhibitor, superior to myricetin in this regard. Future investigations are required to identify the α-glucosidase inhibitors of *D. esculentum. B. orientale, D. esculentum, N. biserrata*, and *P. vittata* also showed cytotoxic effects against K562 cells. Notably, *P. vittata* had the highest cytotoxic activity among the four ferns, with EC_{50} value higher than but still in the same order of magnitude as that of anticancer drug, 5-fluorouracil. This study has provided preliminary but valuable evidence that the edible fern *D. esculentum* is a potential source of an anti-diabetic agent.

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