

Original Research Article

Antioxidant, Iron-chelating and Anti-glucosidase Activities of *Typha domingensis* Pers (Typhaceae)

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Abstract

Purpose: To evaluate the phytochemical profile as well as in vitro antioxidant, iron-chelating, and anti-glucosidase activities of *Typha domingensis* Pers. (Typhaceae)

Methods: Total polyphenols, flavonoids, hydroxycinnamic acids, and pro-anthocyanidins in the respective aqueous extracts of male and female flowers, and fruit of *T. domingensis* were determined by established procedures. Antioxidant activity was evaluated by superoxide anion radical and nitric oxide scavenging assays. Iron chelating activity was assessed using a ferrozine-based assay. Anti-glucosidase activity was determined using 4-nitrophenyl α -D-glucopyranoside as a substrate.

Results: Phenolic contents decreased in the following order: fruit > female flower > male flower. Superoxide scavenging half-maximal effective concentration (EC_{50}) of fruit, female flower and male flower extracts was 3.5, 4.8, and 28.2 mg dry matter (DM)/ml, respectively, while nitric oxide scavenging EC_{50} of fruit, female flower and male flower extracts was 0.16, 0.65, and 0.95 mg DM/ml, respectively. On the other hand, iron chelating EC_{50} of female flower, male flower and fruit extracts was 4.86, 6.43, and 10.88 mg DM/ml, respectively. Only the fruit and female flower extracts exhibited anti-glucosidase activity, with EC_{50} of 0.75 and 5.07 mg DM/ml, respectively.

Conclusion: The fruit and female flower extracts of *T. domingensis* are promising sources of natural antioxidants, iron chelators, and glucosidase inhibitors.

Keywords: *Typha domingensis*, Antioxidants, Iron-chelation, Anti-glucosidase activity.

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INTRODUCTION

The fruits and inflorescences of several *Typha* species are traditionally used to treat wounds and bleeding in different regions of the world [1,2]. The extract of female inflorescence of *T. domingensis* Pers. has been shown to promote wound healing in mice and rats, but the extract of male inflorescence was ineffective [3]. It was hypothesized that the wound-healing effect of the female inflorescence of *T. domingensis* is due to the antioxidant activity of its phenolic constituents [3].

At present, little is known about the antioxidant potential of the female and male flower extracts of *T. domingensis*, neither have their antioxidant activities been compared. Besides antioxidant properties, two other pharmacological properties of *T. domingensis* that are underexplored are iron chelating and glucosidase inhibitory activities. Excess iron in the body is associated with iron-mediated oxidative stress, which increases the risks of neurodegenerative diseases, diabetes, cancer, and stroke [4]. Hence, iron chelators are used as therapeutic agents in the management of iron-related diseases [4]. On the other hand, the

administration of α -glucosidase inhibitors is one of the strategies for managing type 2 diabetes. Glucosidase inhibitors can retard glucose absorption from the small intestine, which in turn decreases the post-prandial rise in blood glucose levels [5].

Various plant-derived phenolic compounds exhibit glucosidase inhibitory activities [5]. Hence, while *T. domingensis* is not traditionally used as an anti-diabetic remedy, its phenolic constituents may possess anti-glucosidase activity. In this study, we sought to compare the antioxidant, iron chelating, and anti-glucosidase potential of the female and male flowers, and the fruit of *T. domingensis*. The contents of bioactive phytochemicals (total polyphenols, flavonoids, hydroxycinnamic acids, and proanthocyanidins) in the extracts were also evaluated.

EXPERIMENTAL

Plant material

The male and female flower inflorescences as well as mature fruits of *Typha domingensis* Pers. were collected in November 2011 from a lake on the Kampar campus of Universiti Tunku Abdul Rahman. The species of the plant was authenticated by H.-C. Ong. A herbarium specimen, designated as TTC11/2011(3), has been stored for future reference at the Department of Chemical Science, Universiti Tunku Abdul Rahman.

Preparation of aqueous extracts

The plant samples were cleaned and then oven-dried at 45°C to constant dry weight. Extracts of the male and female flower inflorescences and the fruit were prepared by mixing pulverized samples with autoclaved deionized water at a 1:20 (dry weight: volume) ratio and incubating the mixture at 90°C for 60 min. The extracts were clarified by vacuum-filtration followed by centrifugation at 9000 rpm and 4°C for 10 min. The supernatant obtained was aliquoted (500 μ l each) and stored at -20°C until used.

Determination of total polyphenols, flavonoids, hydroxycinnamic acids, and proanthocyanidins

Total polyphenol (TP) content of the extracts was determined based on the Folin-Ciocalteu colorimetric method as described in Chai and Wong [6]. TP content was expressed as mg gallic acid equivalents (GAE)/g DM. Total flavonoid (TF) content was determined based on an aluminum chloride colorimetric assay [6]. TF

content was expressed as mg quercetin equivalents (QE)/g DM. Total hydroxycinnamic acid (TH) content was determined using a colorimetric assay with Arnow's reagent [7]. TH content was expressed as mg caffeic acid equivalents (CAE)/g DM, calculated from a standard curve prepared with 0-200 μ g/ml caffeic acid. Total proanthocyanidin (TPR) content was assessed based on the acid-butanol assay [8]. TPR content was calculated with the assumption that effective $E^{1\%, 1\text{ cm}, 550\text{ nm}}$ of leucocyanidin is 460 and expressed as mg leucocyanidin equivalents (LE)/g DM.

Determination of superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity was determined according to the method of Nishikimi *et al.* [9] with minor modifications. Briefly, a mixture of 0.8 ml of potassium phosphate buffer (100 mM, pH 7.4), 0.1 ml of extract, 0.1 ml of 0.78 mM nitroblue tetrazolium, 0.1 ml of 2.34 mM NADH and 50 μ L of 60 μ M phenazine methosulfate were incubated for 20 min at 25 °C in the dark. Thereafter, the absorbance of the mixture was read at 560 nm. A reaction blank was prepared for each measurement by replacing NADH with water. Ascorbic acid was used as the positive control. EC_{50} value, which is the concentration of extract or ascorbic acid required to achieve 50 % scavenging activity in the assay, was determined from the linear range of the plot of radical scavenging activity against extract concentration using linear regression analysis.

Determination of nitric oxide (NO) scavenging activity

NO scavenging activity was determined as previously described [6]. Briefly, a mixture of 0.8 ml of extract and 0.2 ml of sodium nitroprusside (5 mM, in phosphate buffered saline, pH 7.4) was kept at room temperature for 150 min under light source (24-Watt compact fluorescent light bulb). Then, 0.6 ml of the mixture was transferred to a new tube containing 0.6 ml of Griess reagent (1% sulphanilamide and 0.1 % N-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid). This mixture was allowed to stand in darkness for 10 min. Its absorbance was then read at 546 nm. A reaction blank was prepared for each measurement by replacing Griess reagent with water. Ascorbic acid was used as the positive control. NO scavenging activity and EC_{50} values were calculated as described for superoxide anion radical scavenging assay above.

Determination of iron chelating activity

Iron chelating activity of the extracts was assessed based on their ability to interfere with the formation of ferrozine-Fe²⁺ complex *in vitro*. A previously described assay protocol [10] was adopted with minor modifications. Briefly, a mixture of 0.2 ml of 0.10 mM FeSO₄, 0.2 ml of extract, and 0.4 ml of 0.25 mM ferrozine was allowed to react at room temperature for 10 min. Absorbance was then read at 562 nm. To correct for background absorbance, a blank was prepared for each measurement by replacing FeSO₄ and ferrozine with water. Iron chelating activity (R) was calculated as in Eq 1.

$$R (\%) = \{1 - (A_s / A_c)\}100 \dots\dots\dots (1)$$

where A_c is the absorbance of control reaction (without extract) and A_s is the absorbance in the presence of an extract. Disodium salt of EDTA was used as the positive control. EC₅₀ value, defined as the concentration of extract or EDTA required to chelate 50 % of ferrous ions, was computed from the linear range of the plot of iron chelating activity against extract concentration using linear regression analysis.

Determination of glucosidase-inhibitory activity

Glucosidase-inhibitory activity was assessed as previously described [11] with minor modifications. 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 plant 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 minutes. The reaction was terminated by adding 600 μl of 200 mM Na₂CO₃. The absorbance reading was taken at 400 nm. A blank was prepared for each measurement by replacing α-glucosidase with 10 mM phosphate buffer. Anti-glucosidase activity (Ag) was calculated as in Eq 2.

$$Ag (\%) = \{1 - (A_s / A_c)\}100 \dots\dots\dots (2)$$

where A_s is the absorbance of control reaction (without extract) and A_c is the absorbance in the presence of an extract. Quercetin, a flavonoid which is an effective α-glucosidase inhibitor both *in vitro* and *in vivo* [12-14], was used as the positive control. EC₅₀ value, defined as the concentration of extract or quercetin required to achieve 50 % anti-glucosidase activity, was computed from the linear range of the plot of anti-glucosidase activity against extract concentration using linear regression analysis.

Data analysis

All experiments were carried out in triplicates and data reported are mean ± standard error of the mean (SEM). Statistical analyses were performed using SAS (Version 9.2). Data were analyzed by ANOVA test and significant differences between means were separated using Fisher's Least Significant Difference (LSD) test at 0.05 level of probability. Linear regression and correlation analyses were carried out using Microsoft Office Excel 2003.

RESULTS

Total polyphenol, flavonoid, hydroxycinnamic acid, and proanthocyanidin contents

Overall, TP, TF, TH, and TPR contents of the extracts decreased in the following order: fruit > female flower > male flower (Table 1). TP content of fruit extract was about 5-fold higher, while that of the female flower extract was about 2.6-fold higher, compared with male flower extract. TF contents of fruit and female flower extracts were about 13-fold and 4-fold higher, respectively, compared with male flower extract. TH content was 15-fold higher in fruit extract and 5-fold higher in female flower extract in comparison with male flower extract. TPR contents of fruit and female flower extracts were about 29-fold and 7-fold greater, respectively, compared with male flower extract.

Table 1: Total contents (mean ± SEM, n = 3) of polyphenols (TP), flavonoids (TF), hydroxycinnamic acids (TH), and proanthocyanidins (TPR) of the fruit and flower extracts of *T. domingensis*

Extract	TP (mg GAE/g DM)	TF (mg QE/g DM)	TH (mg CAE/g DM)	TPR (mg LE/g DM)
Fruit	51.80 ± 1.15 ^a	103.38 ± 5.29 ^a	52.99 ± 1.78 ^a	36.52 ± 1.13 ^a
Female flower	25.69 ± 0.32 ^b	33.60 ± 1.43 ^b	17.17 ± 0.46 ^b	8.42 ± 0.21 ^b
Male flower	9.92 ± 0.14 ^c	8.16 ± 0.12 ^c	3.46 ± 0.34 ^c	1.28 ± 0.09 ^c

Different superscripts (a-c) denote statistically significant differences within a column. (*p* < 0.05).

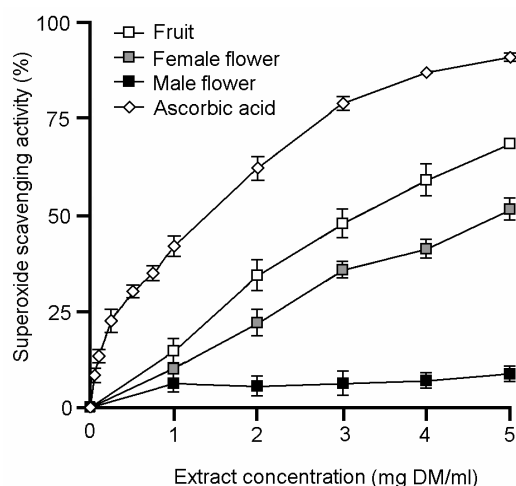


Fig 1: Superoxide anion radical scavenging activities of the fruit and flower extracts of *T. domingensis*, compared with ascorbic acid. Data are mean \pm SEM (n = 3).

Scavenging of superoxide anion radicals and NO

Fruit and female flower extracts both scavenged superoxide anion radicals in a concentration-dependent manner (Fig 1). Male flower extract exhibited relatively low levels of scavenging activity. The EC_{50} values of the extracts were 3.5 (fruit), 4.8 (female flower), and 28.2 mg DM/ml (male flower). The EC_{50} value of ascorbic acid, a positive control, was 1.7 mg/ml.

The male flower extract showed a nearly linear increase in NO scavenging activity within the range of extract concentration tested (Fig 2). In contrast, the fruit extract showed a concentration-dependent NO scavenging activity only up to 0.2 mg DM/ml. The EC_{50} values of the extracts were 0.16 (fruit), 0.65 (female flower), and 0.95 mg DM/ml (male flower). Ascorbic acid, the positive control, had an EC_{50} value of 0.52 mg/ml.

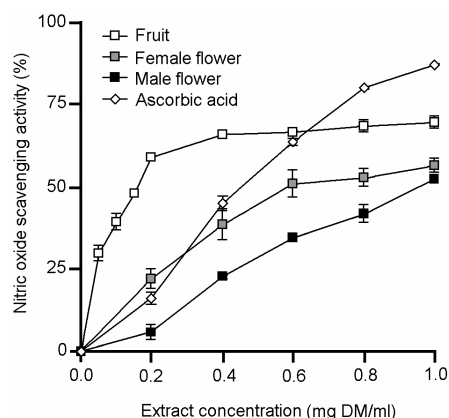


Fig 2: Nitric oxide scavenging activities of the fruit and flower extracts of *T. domingensis*, compared with ascorbic acid. Data are mean \pm SEM (n = 3).

Iron chelating activity

Iron chelating activity of the extracts in descending order was female flower > male flower > fruit (Fig 3). Overall, iron chelating activity rose with increasing extract concentrations. At 10 mg DM/ml, female flower extract exhibited 2-fold greater iron chelating activity, while that of the male flower extract 1.7-fold, compared with fruit extract. The iron chelating activities of the extracts were modest compared with EDTA, the positive control. The EC_{50} values of the extracts were 10.88 (fruit), 4.86 (female flower), and 6.43 mg DM/ml (male flower). The EC_{50} value of EDTA was 0.01 mg/ml.

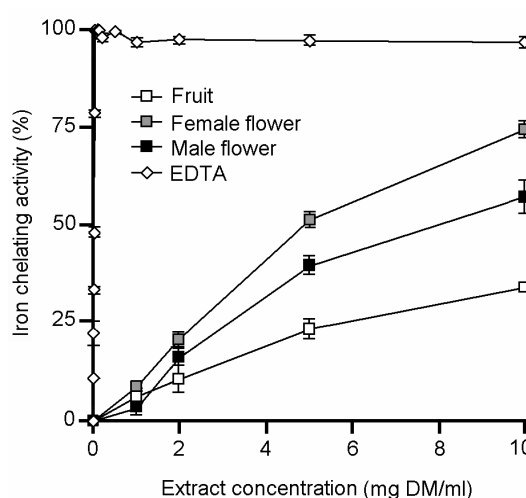


Fig 3: Iron chelating activities of the fruit and flower extracts of *T. domingensis*, compared with EDTA. Data are mean \pm SEM (n = 3).

Glucosidase-inhibitory activity

The fruit extract exhibited the greatest level of glucosidase inhibition among the three extracts, with an EC_{50} value of 0.75 mg DM/ml (Fig 4). The female flower extract showed a moderate anti-glucosidase activity in comparison with fruit extract. EC_{50} value of female flower extract (5.07 mg DM/ml) was about 7-fold higher than that of fruit extract. By contrast, male flower extract exhibited up to 10% glucosidase stimulatory activity over the range of extract concentration tested. The EC_{50} value of quercetin, the positive control, was 0.021 mg/ml.

Correlation analyses

When analyzed collectively, the three extracts exhibited strong, positive correlations between TP contents and free radical (superoxide anion radical and NO) scavenging activities ($R^2=0.81-0.94$, $p < 0.05$). Similarly, TP contents correlated positively with glucosidase inhibitory activity ($R^2 =$

0.79, $p < 0.05$). By contrast, relatively weak correlation was found between TP contents and iron chelating activity ($R^2 = 0.48$, $p < 0.05$). Similar trends were found when correlation analyses were carried out between the bioactivities and levels of other phytochemical contents (TF, TH and TPR) (data not shown).

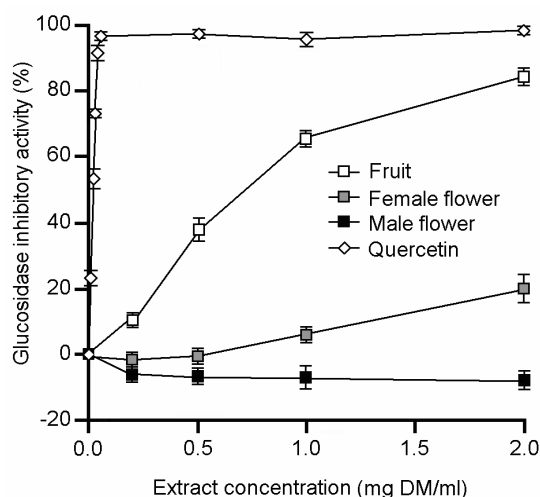


Fig 4: Glucosidase inhibitory activities of the fruit and flower extracts of *T. domingensis*, compared with quercetin. Data are mean \pm SEM ($n = 3$).

DISCUSSION

We demonstrated that the fruit and flower extracts of *T. domingensis* possess different levels of antioxidant, iron chelating and glucosidase inhibitory activities. Correlation analyses suggest that antioxidant and anti-glucosidase activities of the extracts were largely attributable to their phenolic contents. In contrast, the iron chelating activity of the extracts may be contributed by both phenolic and non-phenolic constituents.

The wound-healing effect of the female flower extract of *T. domingensis* has been attributed to the antioxidant activity of phenolic compounds [3]. Our results support the hypothesis in two ways. Firstly, the female flower extract had higher phenolic (TP, TF, TH, and TPR) contents than the male flower extract. Secondly, the female flower extract exhibited greater levels of antioxidant activity (radical scavenging activity and reducing power) compared with the male flower extract. The antioxidant activity of the female flower extract also increased in a concentration-dependent manner. Notably, the female flower extract exhibited higher iron chelating activity than the male flower extract. The iron chelator, deferoxamine has been shown to promote neovascularization and enhance wound healing in diabetic mice [15]. Thus, besides antioxidant activity, iron chelating activity

may also underlie the wound-healing effects of the female flower extract in traditional uses.

The fruit extract of *T. domingensis* had the highest phenolic contents and also the highest antioxidant activity among the three extracts. Considering the beneficial effects of antioxidants in wound healing, the fruit extract may be superior or comparable to the female flower extract as a wound-healing agent. Notably, the fruit extract had a higher proanthocyanidin content than the female flower extract. It has been reported that grape seed proanthocyanidin extract has wound-healing effects in animal models [16].

Our study suggests that the fruit and female flower extracts of *T. domingensis* contained phenolic compounds with glucosidase inhibitory activity. These extracts may be developed into anti-hyperglycemic agents with concurrent iron chelating and antioxidant activities. Iron chelating and antioxidant activities may add to the potency of the extracts as anti-diabetic agent. Notably, iron chelation has been shown to prevent diabetes as well as preserving the function of insulin-producing pancreatic beta cells in diabetes-prone obese mice [17]. In addition, iron chelation therapy improves glycemic control in iron-induced diabetes and prevents or retards the progression of diabetic complications [18]. On the other hand, an anti-hyperglycemic agent with antioxidant properties may have additional therapeutic benefits as oxidative stress is involved in the pathogenesis of diabetes and its complications [19].

CONCLUSION

This study demonstrates that *T. domingensis* is a good source of natural antioxidants, iron chelators, and glucosidase inhibitors. The fruit extract of the plant is a superior antioxidant and anti-glucosidase agent to the male and female flower extracts. The female flower extract, by contrast, is the most potent iron chelating agent among the three extracts. Both antioxidant and anti-glucosidase activities can be attributed to the phenolic contents of the extracts. Our findings provide a biochemical rationale for the wound-healing effects and anti-diabetic potential of *T. domingensis*.

Further studies are required to evaluate the anti-hyperglycemic effects of *T. domingensis* *in vivo*. In addition, future work should isolate and identify the active principles of *T. domingensis*, specifically, the fruit and female flower.

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