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PHENOLIC CONTENT, ANTI-OXIDATIVE, ANTI-α-AMYLASE AND ANTI-α-GLUCOSIDASE ACTIVITIES OF *SOLANUM DIPHYLLUM* L.

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Abstract

Total phenolic content, anti-oxidative, anti-α-amylase and anti-α-glucosidase activities in the powder extracts of fruits, leaves, roots and stems of *Solanum diphyllum* were determined. Leaf extract had the highest total polyphenol content (68.1 mg GAE/g), followed by stems (48.6 mg GAE/g), fruits (38 mg GAE/g) and roots (26 mg GAE/g). By employing different assays such as DPPH radical scavenging, reducing power, Fe^{2+} chelating and total anti-oxidant capacity, it was found that leaf and stem extracts had promising anti-oxidative activity. Stem extract had highest level of α-amylase and α-glucosidase inhibition at concentrations of 1.0 and 0.5 mg/ml, respectively.

Introduction

Many of the healthful properties of plants are attributed to the anti-oxidative activities of their constituents including polyphenols and vitamins. Anti-oxidants inhibit lipid peroxidation, scavenge free radicals and chelate divalent cations (Shon *et al.* 2004). Anti-oxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and some of the associated complications. Especially, phenolic compounds, such as catechin and its derivatives have a wide range of health promoting effects (Hossain *et al.* 2002, 2007).

Solanum diphyllum L. is a small shrub 1-2 m tall with glabrous, oblanceolate leaves, and the fruits are globose berry. This plant is native to Mexico and Central America and also distributed in many tropical and subtropical countries, and is used as foliage shrub. No scientific data addressing its biological activities are available. The present research is aimed at investigating and recording the polyphenol content, anti-oxidative, anti-α-amylase and anti-α-glucosidase activities of the fruits, leaves, roots and stems of this wild plant.

Materials and Methods

Plant collection and extraction: Mature fruiting *S. diphyllum* plants were collected and fruits, leaves, roots and stems were separated and air dried at room temperature. The voucher samples were deposited in the herbarium of Aswan Faculty of Science, South Valley University, Egypt. The dried plant materials were then powdered and defatted with n-hexane to remove non-polar compounds and then the defatted materials were extracted with ethanol. Ethanol extract of fruits, leaves, roots and stems of the plants were denoted as SDF, SDL, SDR and SDS, respectively. To conduct experiments, 20 mg of dry ethanol extract was dissolved in 1 ml of ethanol.

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Determination of total phenolics (TPH): The TPH in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1988) with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract (Hossain *et al.* 2008).

DPPH radical scavenging activity: The reaction mixture (total volume 3 ml), consisting of 0.5 ml of a 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 1.5 mL of a 50% (v/v) ethanol aqueous solution, was shaken vigorously with the extracts (Blois 1958). After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm (Hossain *et al.* 2008).

Reducing power activity: The reducing power of the extracts was determined according to the method of (Oyaizu 1986).

Measurement of chelating activity: The activity of extracts to chelate Fe^{2+} was measured according to the method of Carter (1971).

Determination of total anti-oxidant capacity: The assay was done according to Prieto *et al.* (1999).

α-Amylase assays in vitro: α-Amylase activity was carried out using the starch-iodine method (Hossain *et al.* 2008, 2009).

α-Glucosidase assays in vitro: Different concentrations of extract was mixed with α-Glucosidase and incubated at 37º C for 10 min. Hereafter, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was added to the mixture to start the reaction. The reaction mixture was incubated at 37º C for 60 min and stopped by adding 2.5 ml of 0.1 M Na_2CO_3. The α-Glucosidase activity was determined by measuring the absorbance at 400 nm.

Results and Discussion

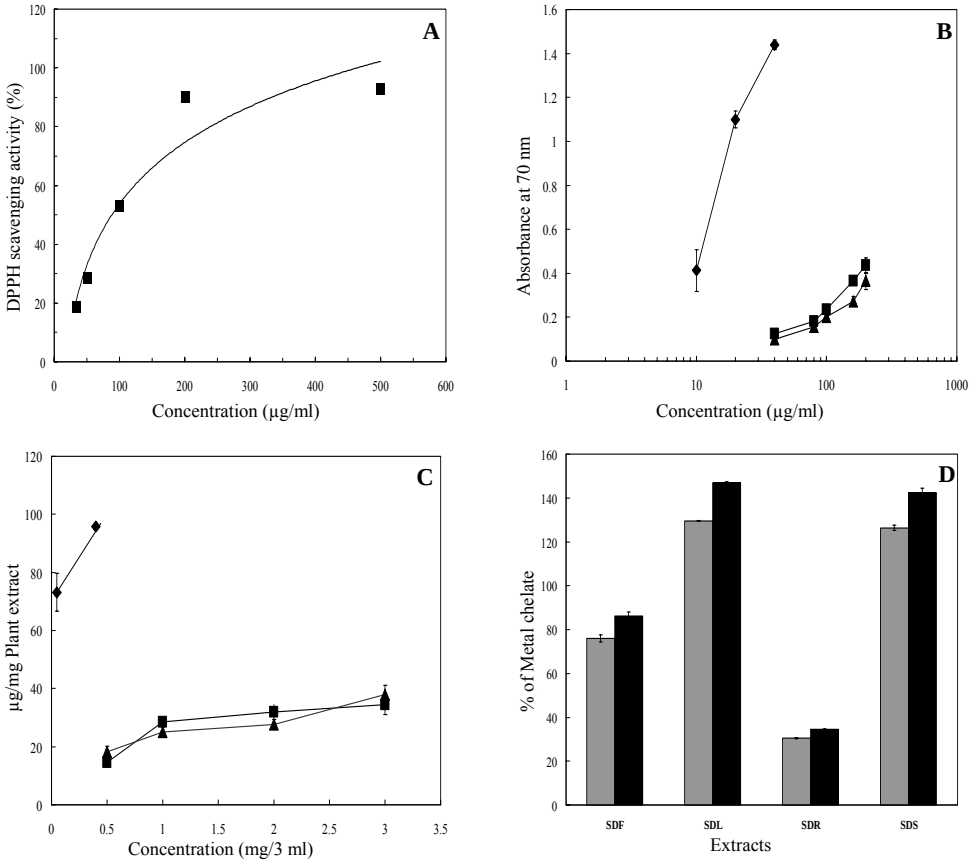
Total phenolics (TPH) and anti-oxidant activity: The TPH content of the different extracts ranged from 68.1 to 26 mg GAE/g of extract (Table 1). The amount was largest in SDL (68.1 mg GAE/g) followed by SDS (48.6 mg GAE/g). The smallest amount was in SDR (26 mg GAE/g) followed by SDF (38 mg GAE/g). The DPPH radical scavenging activities of the different extracts are shown in Table 1. At 100 µg/ml, SDL showed the highest DPPH radical scavenging activity (53.2%). Fig. 1A shows the dose-dependent DPPH radical scavenging activity of SDL with IC_{50} value of 93.5 µg/ml. The reducing power of the extracts determined using the potassium ferricyanide reduction method is shown in Table 1. Since SDL and SDS had the highest levels of reducing activity among the extracts, their dose-dependent increase of reducing power was

Table 1. Total phenolic content, DPPH radical scavenging activity, reducing power and metal chelating activity of the ethanol extracts from parts of *Solanum diphyllum*. n = 3 or 5, ± = s.d.

Extracts	Total phenolic content (mg GAE/g extract)	DPPH radical scavenging activity (% at 0.1 mg/ml)	Reducing power (OD. at 0.1 mg/ml)	Metal chelating activity (% at 2.0 mg/3ml)
SDF	38.0 ± 0.2	37.5 ± 1.8	0.12 ± 0.01	-
SDL	68.1 ± 1.8	53.2 ± 1.2	0.24 ± 0.01	31.9 ± 2.5
SDR	26.0 ± 0.3	40.3 ± 0.7	0.15 ± 0.01	3.1 ± 0.7*
SDS	48.6 ± 0.5	42.6 ± 1.0	0.20 ± 0.01	27.6 ± 0.5

$p < 0.01$ by Student's *t* test for values between the sample and the control in DPPH, and metal chelating experiments. *not significant.

examined (Fig. 1B). Table 1 shows the chelating effect of the extracts on ferrous ions. Among the extracts, SDL and SDS were the most promising chelators, and their dose-dependent activities were examined (Fig. 1C). SDL and SDS exhibited chelating effects on ferrous ions, suggesting that they minimize the concentration of metal in the Fenton reaction. Total anti-oxidant capacities, which are expressed as the ascorbic acid (AAE) and gallic acid (GAE) equivalents of the different extract are shown in Fig. 1D. The capacity was highest in leaf followed by stem, whereas the lowest in roots. In this study, extract with the high proportion of polyphenols, displayed high anti-oxidant activity, particularly in case of leaf and stem. SDL and SDS extracts displayed a remarkable capacity to bind iron, suggesting that they may protect against peroxidation. An affinity for ferrous ions minimizes the concentration of the catalyzing transition metal needed in a lipid peroxidation reaction. Owing to the complexity of the oxidation-anti-oxidation process, no single testing method is capable of providing a comprehensive view of the anti-oxidative profile of a sample (Parejo *et al.* 2002). Therefore, multi-method approach is necessary to assess anti-oxidative activity.



Figs 1A-D. Anti-oxidative activity of the extracts. A. Dose-dependency of the DPPH free radical scavenging activity of SDL; B. Dose-dependent increase of reducing power of SDL (■) and SDS (▲) {ascorbic acid (◆) as positive control}; C. Metal (Fe^{2+}) chelating ability of SDL (■) and SDS (▲) at different concentrations {EDTA (◆) as positive control}; D. Comparison of total anti-oxidative capacity of various extracts {AAE (□), GAE (■)}. n = 3 or 5, ± = s.d.

Anti-α-amylase and anti-α-glucosidase activities: Among the extracts, SDS strongly inhibited ($p < 0.01$) both α-amylase, and α-glucosidase activities (Fig. 2). It also showed dose-dependent increase in α-amylase inhibitory activity. At the concentrations of 500, 1000 and 2000 μg/ml, SDS showed α-amylase inhibitory activity of 20.3 ± 0.4, 28.0 ± 0.3 and 38.8 ± 3.6%, respectively. Concentrations more than 2000 μg/ml showed no further inhibitory effect. Increasing the concentration of starch 1% to 5% had no effects on the inhibitory activity, indicating non-competitive inhibition. Since, inhibition of α-glucosidase was not promising by the extracts no further experiment was conducted on it. Natural products that reduce post-prandial hyper-glycaemia by suppressing the hydrolysis of carbohydrates may be helpful in the control of diabetes mellitus. In the present study, activities of α-amylase and α-glucosidase were significantly ($p < 0.01$) inhibited by SDS (Fig. 2). In dose dependent effects on α-amylase, at high concentrations probably there were saturation of component(s) thereby causing no further increase

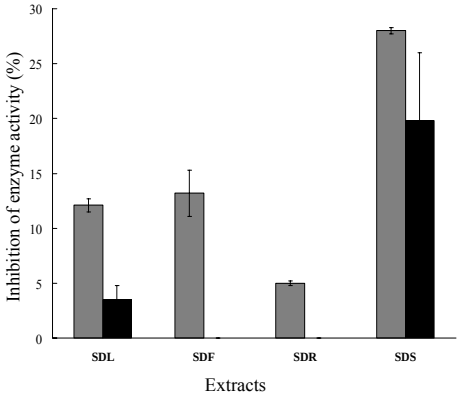


Fig. 2. Effects of various extracts on the inhibition of α-amylase (□), and α-glucosidase (■) activity (%). Enzyme without any extract was taken to be 100% activity (control). Inhibition (%) of the α-amylase activity was studied in presence of 1 mg/ml extract and that for α-glucosidase was 0.5 mg/ml. $n = 3$ or 5, ± = s.d.

in inhibition. This extract probably non-competitively binds to the active site of the enzyme. Promising anti-oxidative, anti-α-amylase and iron-chelating activities of SDS indicate that it might be used to prevent onset of various diseases including diabetes, cancer, inflammation, etc. Various natural products inhibit α-amylase and α-glucosidase activities (Hossain *et al.* 2008, 2009) such as flavone, flavonoids, etc. (Havsteen 1983, Kim *et al.* 2000). Moreover, polyphenols also have anti-hyperglycemic effects (Hossain *et al.* 2002, Hanamura *et al.* 2006), and inhibit the development of diabetes (Zunino *et al.* 2007).

Attention should be given especially to SDL and SDS to search out natural anti-oxidant(s), and α-amylase inhibitor(s). Moreover, fractionation of the extracts is essential to know the phenolic or nonphenolic compound(s) responsible for the anti-oxidative and anti-amylase activities. It is also necessary to elucidate potential cytotoxic effects, since some polyphenols perturb the membrane structure (Hossain *et al.* 2002, Aoshima *et al.* 2005).

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