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Assessment of *in vitro* antioxidant activity study of polydatin

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Abstract
Polydatin is a monocrystalline segregated from polygonum cuspidatum sieb et zucc. Polydatin also named piceid (3,4',5-trihydroxystilbene-3-β-D glucoside,PD).Polydatin is also detected in grapes, peanut, hopcons, redwines and hop pellets. Umpteen pharmacological exploration of polydatin mainly focus on cardiovascular effects, neuroprotection, immunoregulatory effects, anti –inflammatory, anti-oxidation, anti-tumor [1]. In our present study was to evaluate the antioxidant activity of polydatin. Antioxidant activity of polydatin determined by four different invitromethods.Theyare1,1diphenyl-2-picrylhydrazlradical(DPPH·),2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS·),hydroxyl radical (OH·) and superoxide anion radical (O_2·⁻) established the free radical scavenging and antioxidant activity of polydatin [2].

Keywords: Polydatin, antioxidant activity, free radical

Introduction
Free radical is a molecule which contain unpaired electrons in an outer orbital. Free radicals are highly unstable and highly reactive. Free radicals are normally generated in our body as a part of the normal metabolic process. They play a twin role in our body as both detrimental and beneficial effects [3]. At high concentration of reactive oxygen species (ROS) are highly reactive and toxic causing damage to cell membrane, proteins, lipids and DNA leading to oxidative stress. This oxidative stress cause tissue damage result in many diseases such as cardiovascular disease, diabetes, cancer, neurodegenerative disease, aging, rhomatoidarthritis and cataract [4]. Antioxidant play important role in protectingour body against free radical damage. They balance the production of free radicals and detoxify them when in excess [5]. Antioxidants are categorized in two groups of synthetic and natural. Natural antioxidants present in fruits and vegetables [6]. The toxic effects of reactive oxygen species opposed by enzymatic as well as non-enzymatic antioxidants system such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbicacid, Tocopheral, Glutathione, carotenoids and flavonoids neutralize the effects of reactive oxygen species and thus help in preventing diseases [7]. The polydatin have the antioxidant activity which might be helpful to protect the body from oxidation. The aim of this work was to estimate the antioxidant activity of polydatin.

Materials and methods
Polydatin is available as off white powder having molecular weight 390.38.This drug was purchased from SIGMA-ALDRICH, Germany. The polydatin was soluble in ethanol and dimethl sulfoxide (DMSO).

DPPH Scavenging assay
The DPPH radical scavenging activity was determined according to the method of Blois (1958) [8]. DPPH solution was prepared at the concentrationn of 0.1mM in methanol. During the assay, 1mL of test solution (20-120µg/mL) was mixed with 1mL of DPPH solution. The homogenous was incubated at room temperature in dark for 30 minutes. The absorbance was recorded at 517nm by uv visible spectrophotometer. The percentage of DPPH free radical scavenging activity was calculated by following formula.
RSA% = [(Abs (control)-Abs (sample)/Abs (control)] ×100
A indicates the absorbance value

Table 1: Percentage of DPPH free radical scavenging activity

DPPH Activity	OD @ 517 nm	%
Control	0.396	
20	0.184	53.53
40	0.152	61.61
60	0.113	71.46
80	0.091	77.02
100	0.076	80.8
120	0.042	88.63

Table 2: The percentage of hydroxyl radical scavenging activity

Hydroxyl	OD @ 520nm	%
Control	0.274	
20	0.213	22.26
40	0.208	24.08
60	0.2	27
80	0.17	37.95
100	0.154	43.79
120	0.122	55.47

Table 3: The Percentage of ABTS$^{\bullet+}$ radical cation scavenging activity

ABTS	OD @ 734nm	%
Control	0.172	
20	0.114	33.72
40	0.103	40.11
60	0.093	45.93
80	0.077	55.23
100	0.056	67.44
120	0.028	83.72

Table 4: The percentage of superoxide radical scavenging activity

Superoxide	OD @ 590nm	%
Control	0.283	
20	0.174	38.51
40	0.169	40.28
60	0.138	51.23
80	0.111	60.77
100	0.102	63.95
120	0.085	69.96

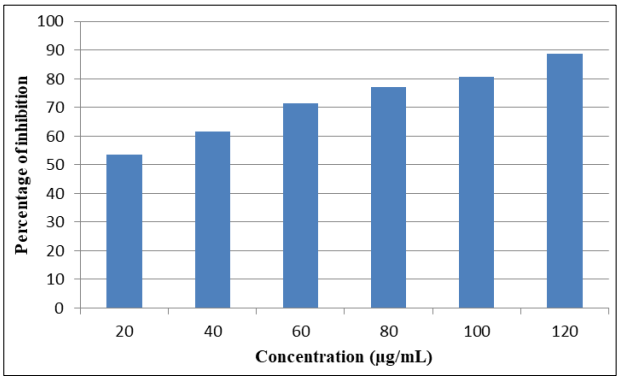

Fig 1: DPPH assay

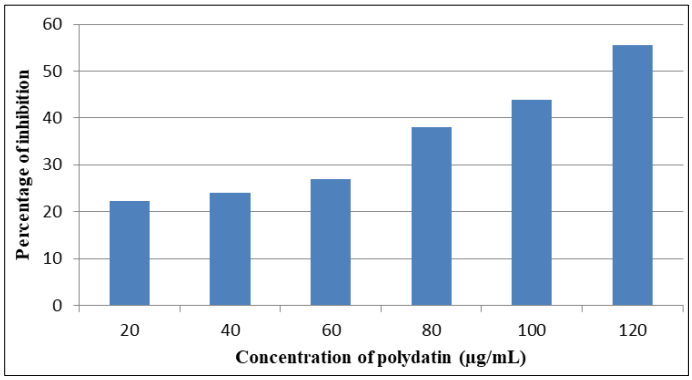

Fig 2: Hydroxyl radical scavenging assay

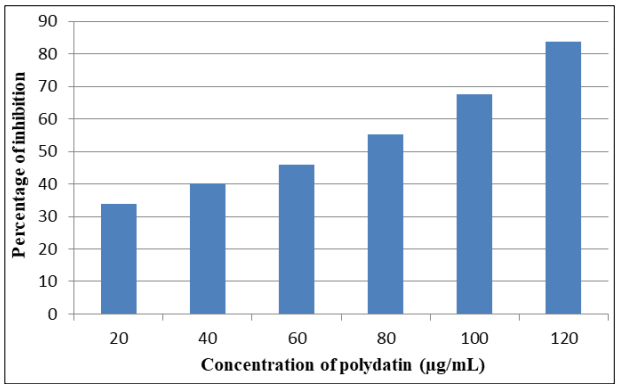

Fig 3: ABTS Radical Scavenging Assay

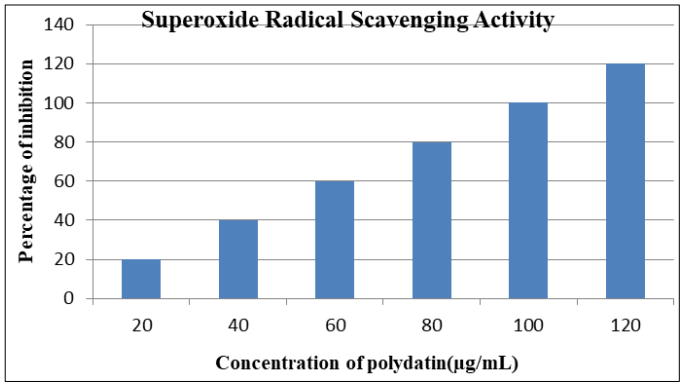

Fig 4: Superoxide anion scavenging assay

Hydroxyl radical scavenging activity [Klein *et al*. 1981]

Hydroxy radical scavenging activities of the test sample (20-120µg/mL) were estimated by the method of Klein *et al*. 1981 [9]. Various concentrations of the samples were added with 1ml of iron–EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA),0.5mL of 0.018% EDTA and 1ml of 0.85% (v/v) DMSO (in 0.1 M phosphate buffer, pH -7.4) were added followed by 0.5mL of 0.22% (w/v) ascorbic acid. The tubes were closed tightly and incubated on water bath at 85 ºC for 15 minutes. In post incubation the test tubes were uncapped and ice – cold trichloroaceticacid (17.5% w/v) was added in each tube immediately. Add 3 mL OF Nash reagent (7.5 g of ammonium acetate, 300µL glacial acetic acid and 200µL acetyl acetone were mixed and made upto 100 mL with distilled water to all the tubes and incubated at room temperature for 15 minutes. Absorbance was read at 412 nm using UV visible spectrophotometer. Percentage hydroxyl radical scavenging activity (HRSA %) was determined by the following formula as given below.

HRSA% = [(Abs (control)-Abs (sample)/Abs (control)]×100

Where A denotes the absorbance.

ABTS$^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was estimated in terms of the ABTS$^{\bullet+}$ 'radical cation scavenging ability was determined using a spectrophotometer (Re *et al*. 1998) [10]. ABTS$^{\bullet+}$ was obtained by 7 mM of ABTS stock solution with2-45 min potassium persulfate and the mixture was left to stand in the dark room temperature for 12-15 hours before use. The ABTS$^{\bullet+}$ solution was kept stable for two days and diluted with distilled water to reach an absorbance of 0.70±0.02 at

734 nm. Divergent concentration of test sample (5-30µg/mL) was mixed with 500µL of diluted ABTS solution and the absorbance was measured at 734nm after 10 minutes. The $ABTS^{\bullet+}$ radical scavenging activity was expressed as % of $ABTS^{\bullet+}$ radical cation inhibition = [(Abs (control)-Abs (sample)/Abs (control)]× 100

Superoxide (O2.-) radical scavenging activity

Superoxide radical scavenging activity executed by the method of winterbone *et al*. 1975 [11]. The reaction mixture hold in different concentration (20-120µg/mL) of test sample, 50mM of phosphate buffer (PH – 7.8) 1.5 mM of riboflavin 12 mM of EDTA and 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 150 s. Immediately after the process of illumination the absorbance was measured at 590nm.

% Superoxide (O_2^{-}) radical = [Abs (control) – Abs (sample) /Abs control]×100

A denotes the absorbance value

Result and discussion

The effect of antioxidant on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen atom to become a stable diamagnetic molecule [12]. DPPH is a purple coloured free radical. When a solution of DPPH mixed with a substance that can donate hydrogen atom then it was reduced into the yellow–coloureddiphenylpicrylhydrazine which was measured at spectrophotometric ally [13]. The percentage of inhibition shown in table 1.

The calibration curve of DPPH radical scavenging activity of polydatin was observed and shown in fig 1. The free radical scavenging was found to be overwhelm the concentration of the drug increases. Hydroxyl radical is a highly reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moities of cell membrane phospholipids and cause damage to cell. The hydroxyl radical scavenging ability of polydatin established highest radical scavenging activity [14]. Polydatin can be used as a good hydroxyl radical scavenger. The percentage of hydroxyl radical scavenging activity of polydatin was shown in table 2.

The dose response curve of hydroxyl radical scavenging assay of polydatin was presented in fig 2.The polydatin contain sustainable hydroxyl radical scavenging activity.

The ABTS cation radical which absorbs at 734 nm is formed by the loss of an electron by the nitrogen atom of ABTS. ABTS can be oxidized by potassium persulphate giving rise to the ABTS cation radical, then it was reduced in the presence of the sample composite, polydatin a hydrogen donating antioxidant [15]. The antioxidant activity was estimated by plotting percentage inhibition against diverse concentration of compound. The percentage inhibition shown in table 3. The calibration curve of ABTS scavenging activity of polydatin behaviour was observed and illustrate in fig 3.

Superoxide radical was generated from the photo reduction of riboflavin and was detected by NBT reduction method. Superoxide is a highly reactive molecule that reacts with other substance produd by metabolic process [16]. The percentage inhibition values shown in table 4.The percentage of superoxide anion scavenging activity of polydatin was illustrated in fig 4. The polydatin exhibited strong superoxide radical scavenging activity.

Conclusion

The aim of this work the antioxidant activity of polydatin was examined. Antioxidant play an important role in inhibiting and scavenging radicals, thus providing protection to humans and against infections and degenerative diseases. For further study we will do the anti-cancer, anti-inflammatory, anti-diabetic and myocardial activity because of polydatin exhibited good antioxidant activity.

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