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Dose dependency of anti-oxidant activity of quercetin glycosides of *Annona cherimola*

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ABSTRACT

Aim: Quercetin glycosides (flavonoids) isolated from methanol extract of leaves of *Annona cherimola* is found showing prominent anti-oxidant activity. The objective is to evaluate the dose dependency of anti-oxidant activity of the three quercetin glycosides isolated.

Method: The dose response of anti-oxidant potential IC₅₀ of the isolates against superoxide, DPPH and ABTS radicals was carried out by using standard experimental methods.

Results: The studies revealed that the dose response is linear with R² values ranging from 0.9 to 1.0 in all the cases. The activity of quercetin arabinopyranoside is found more prominent than the quercetin glycosides of galactose and glucose.

Conclusion: The anti-oxidant potential of the three quercetin glycosides isolated from *A.cherimola* is found dose dependent and the response is linear.

Keywords: anti-oxidant potential, superoxide radical, DPPH radical, ABTS radical

INTRODUCTION

Chemical diversity in plants serves as a rich source of medicinal products because of their precious therapeutic activities and also low toxicity profile. In plants there are a wide variety of naturally occurring anti-oxidants, which are different in their composition, physical and chemical properties, mechanism and site of action. Hence screening of different plant extracts is being done by measuring the anti-oxidant activity through various *in vitro* and *in vivo* models. Through preliminary phytochemical screening the plant *Annona cherimola* was reported to contain alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins phenolic compounds, phytosterols and amino acids [1, 2]. A study was carried out to isolate the constituents of leaves of *A.cherimola* and elucidated the structures of them by using spectroscopic methods and also evaluated their pharmacological activity by both *in vivo* and *in vitro* methods [1, 2, 3]. On identifying the anti-oxidant and anti-inflammatory potential of the chemical constituents isolated from the methanol

extract of leaves of *A.cherimola* [4], further investigations are conducted to trace the effect of dose variation on the anti-oxidant activity of the three quercetin glycosides (flavonoids) isolated (figures: 1, 2 & 3).

Figure.1. Structure of Quercetin-3-O-galactopyranoside

Figure.2. Structure of Quercetin-3-O-glucopyranoside

Figure.3. Structure of Quercetin-3-O-arabinopyranoside

METHODS

In view of the structures of the isolated compounds of *A. cherimola*, especially the presence of hydroxyl groups, the dose dependency of anti-oxidant activity studies of the compounds was carried out by the following *in vitro* methods.

Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of various isolates of *A. cherimola* extracts was determined by Nitro Blue Tetrazolium (NBT) riboflavin photo reduction method of McCord and Fridovich [5, 6]. The assay mixture contained EDTA solution (6.6mM) containing NaCN (3μg), riboflavin (2μM), NBT (50μM), test substances and phosphate buffer (67mM, pH 7.8) in a final volume of 3 mL. The absorbance at 560 nm was measured before and 15 min after illumination. All tests were run in triplicate and mean values were used to calculate the percentage scavenging ability and IC₅₀ values were calculated using linear regression analysis.

DPPH Free Radical Scavenging Activity

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity of the test compounds was determined by the method of Szabo *et al.* [7]. The reaction mixture contained 1.5×10^{-7} M methanolic solution of DPPH and various concentrations of isolates of *A. cherimola* extract and were kept in dark for 50 min. Optical density (OD) of the samples was measured at 517 nm against a blank and IC₅₀ values were calculated using linear regression analysis.

ABTS Anti-oxidant Assay

The ABTS assay of ReRoberta *et al.* [8, 9] was employed to measure the anti-oxidant activity of isolates of *A. cherimola* extracts.

ABTS was dissolved in distilled water to 7mM concentration and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12-16 h) in dark before usage. The resultant intensely colored ABTS^{•+} radical cation was diluted with ethanol to give an absorbance value of 0.70 (approximately) at 734 nM. Various concentrations of *A. cherimola* were incubated with the ABTS^{•+} solution for 30 min and OD was measured at 734 nM against a blank and IC₅₀ values were calculated using linear regression analysis.

RESULTS

The results of scavenging activity of isolates of *A. cherimola* are summarized in Table no 1. The activity was compared with that of gallic acid and vitamin C. The IC₅₀ values of the three quercetin glycosides, and the percentage inhibition with variation in dose clearly shows the prominent anti-oxidant potential of the compounds. The linear relationship between dose and percentage inhibition is clear from the graphs.

DISCUSSION

Flavonoids are diphenylpropanoids and they demonstrate a wide spectrum of biological activity [10]. The isolated quercetin glycosides which are flavonoids have the ability to act as anti-oxidants by a free radical scavenging mechanism with the formation of less reactive flavonoids phenoxyl radicals. The high potential of flavonoid compounds to scavenge free radicals may be explained by the presence of 3,4-dihydroxy i.e. *O*-dihydroxy group (catechol structure) in the B ring having electron donating property, and C₃-C₄ double bond conjugated with 4-keto group, which is responsible for electron delocalization from B

Table No 1: Dose dependency of scavenging activity of quercetin glycosides

Radical used	test substance	dose ($\mu\text{g}/\text{ml}$)	percentage inhibition	dose response	IC ₅₀ ($\mu\text{g}/\text{ml}$)
superoxide radical scavenging activity	Quercetin galactopyranoside	12.5	24.7		27.35
		25	53.51		
		50	79.4		
	Quercetin glucopyranoside	12.5	21.57		34.22
		25	46.71		
		50	65.05		
	quercetin arabinopyranoside	6.25	26		13.6
		12.5	52.64		
		25	79.5		
	gallic acid (standard)	0.25	32.23		0.52 (current)
		0.5	50.76		
		0.75	63.07		
DPPH radical scavenging activity	Quercetin galactopyranoside	2.5	31.32		5.98
		5	44.75		
		10	71.55		
	Quercetin glucopyranoside	2.5	19.1		9.91
		5	30.52		
		10	54.21		
	quercetin arabinopyranoside	1	22.88		2.87
		2.5	45.43		
		5	79.87		
	vitamin C(standard)	1	18.06		3.01 (current)
		2.5	42.25		
		5	81.37		
ABTS radical scavenging activity	Quercetin galactopyranoside	2.5	31.4		5.67
		5	48.45		
		10	72.76		
	Quercetin glucopyranoside	2.5	26.73		7.39
		5	39.77		
		10	61.84		
	quercetin arabinopyranoside	1	27.1		2.62
		2.5	50		
		5	81.63		
	vitamin C(standard)	1	36.52		1.63 (current)
		2.5	69.68		
		5	97.68		

ring, enhancing further the radical scavenging capacity. And also, the presence of 5-OH group in combination with a 4-Carbonyl functional group and C2-C₃ double bond further increases the radical scavenging activity of the flavonoid [11]. The relationship between concentration of test substances and the percentage inhibition is found to be linear in all the assays investigated. Concentration dependent free radical scavenging activity of extracts of *A. cherimola* fruits was also earlier reported [1]. Among the quercetin glycosides the activity is found in the order: Quercetin arabinopyranoside > Quercetin galactopyranoside > Quercetin glucopyranoside. It is reported that polarity [12] and molecular weight [13] of the compounds had a significant effect on the anti-oxidant activity of them.

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CONFLICT OF INTEREST: Nil

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