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Gallic acid Substance Appraisal in *Daucus carota sp. sativus* and genus Vitis by Conventional Withdrawal and High Performance Liquid Chromatography

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ABSTRACT

Gallic acid(GA) contains three hydroxy groups, which are catalyzed by enzymes during the methylation or sulphation reactions. The present research portrays GA substance appraisal in a root vegetable Carrot (*Daucus carota sp. sativus*) and botanically a berry like grapes (genus Vitis). Conventional withdrawal process was performed by using methanol as solvent followed by maceration and distillation process. The study sought to quantify and compare the contribution of GA in Carrot and grapes for the nutritional care of society. The determination of GA content was quantified using HPLC (High performance liquid chromatography) method with. Zodiac C18(250mmx 4.6mm, 5µm) column. The other chromatographic conditions applied are detection at 280nm and flow rate 1mL/ minute. The detection limit (0.11–0.9 µg/mL) and quantification limit (0.9–3.0 µg/mL) were obtained. The reported method was validated and has the advantage of being fast, simple, and accurate.

Keywords: Gallic acid, Extraction, Characterization, *Daucus carota sp. sativus*, genus Vitis, High performance liquid chromatography.

INTRODUCTION

Gallic acid (GA) is also known as (3, 4, 5-tri hydroxy benzoic acid) the formula is C7H605. GA is naturally occurring polyphenolic compound present in fruits, vegetables and edible parts of plants. GA is a white solid, soluble in water, alcohol and melt at 235-240°C. GA is a secondary polyphenolic metabolite in various vegetables and fruits and is a bioactive component of natural antioxidants. GA bio efficacy impact has been the subject of many research papers and reviews to date. GA has many biological properties[1] which includes antioxidant[2-3], anticancer property[4-5], anti-inflammatory[6], antidiabetic[7-8], anti-ulcerogenic[9]. GA is also safe against disorders like radical oxygen species (ROS), hyper production, oxidative stress (OS)[10] and bacterial infections[11]. GA and its derivatives have the potential to be innovative therapeutic and preventative medicines for gastrointestinal illnesses[12]. GA is phenolic acid which is derived from shikimic acid, it is basically a secondary polyphenolic metabolite. Phenolic compound plays a crucial role in maintaining a healthy society. Many bad dietary habits nowadays

lower food nutritional contents[13], and an insufficient consumption of nutritious food causes a large dietary imbalance, which is a leading cause of chronic diseases. The synthesis and concentrations of GA were determined using HPLC[14-16]. Fig. 1 shows the chemical structure of GA. The interaction of GA with polysaccharide can boost its value as an antioxidant in the human being[17-19]. Keeping in view of a breeding interest in food based approaches for chronic disease prevention so the author reported the extraction and characterization of GA were determined in carrot and grape using HPLC-UV Method.

Chemicals and reagents

GA(standard) from Sigma-Aldrich, HPLC-grade water, Ortho -phosphoric acid Methanol, Acetonitrile (85%), Ammonium acetate-(Merck) India.

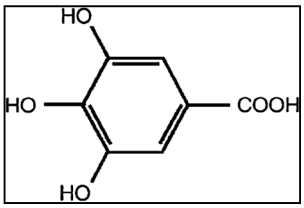

Fig. 1. Structure of GA

Plant materials

Cultivators bought nutritious fresh fruits and vegetables (grape and carrot) from the local market in Andhra Pradesh, India. For storage of all the specified fruits and vegetables, clean and dried glass containers were utilized, and they were left at room temperature. Each kilogram of chosen fruit and vegetable substance acquired from cultivators was left to dry and scrubbed.

Preparation of sample and standard solution

The preselected materials had been slashed into small pieces or bits and positioned on a spotless filter paper. A potable grinding machine is used to finely ground the plant material. After drying, 100 g of each sample was soaked for 30 min in small portions of (1:20) Methanol and 1:1 aqueous Hydrochloric acid solution in an orbital shaking incubator for one hour.

The components were cooled, screened, and the solution was filtered had been dried in a rotary vacuum evaporator at 40°C to obtain a dense concentrate sample, which was then stored for analysis.

Standard solution and test solution preparation

10mg of GA standard was taken in a 50.0 mL volumetric flask and 5 mL of methanol was added to it followed by sonication.

Column chromatography conditions

HPLC (Agilent Technologies, 1260) with Zodiac C18 column, 250mmx4.6mm, 5micrometer was used. The chromatographic conditions applied are Detector-UV, diluent-mobile phase, 10micro liter-injection volume & wavelength-280nm, Chromatogram run time:20 min, Column and temperature cooling 35°C and 10°C, Isocratic pump mode and 1.0 mL/min flow rate , Methanol and water in a 700:300v/v ratio with 1.0 mL Formic acid was selected as mobile phase.

RESULTS AND DISCUSSIONS

The secondary polyphenolic metabolite GA is used to reduce ulcer, inflammatory disease, blood glucose levels, viral infections, and so on. Fruits and vegetables have a high GA content which is essential to cure diseases. Selected mobile phase with 1.0 milliliters of 1% formic acid, mix 700:300v/v methanol and water were used. A perfect baseline analyte resolution is shown in Fig. 2-5 show chromatograms of GA. Table 1 shows the GA content of selected grape and carrot samples.

Table 1: GA in grape and Carrot

Fruit/Vegetable	S1	S2	S3	S4	S5
Grape white(mg/100g)	0.281	0.361	0.279	0.452	0.618
Carrot root vegetable(mg/100g)	0.658	0.698	0.714	0.874	0.657

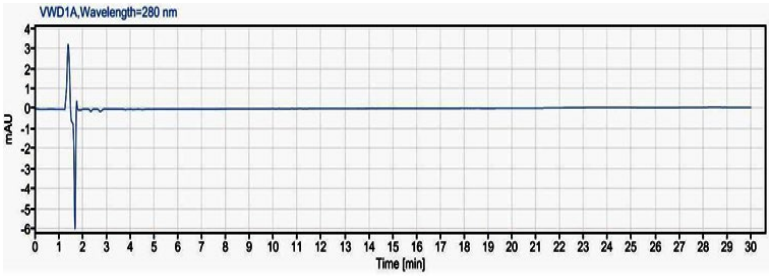

Fig. 2. Blank chromatogram

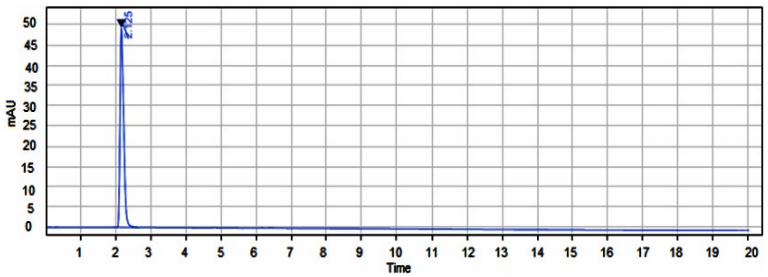

Fig. 3. GA Standard chromatogram

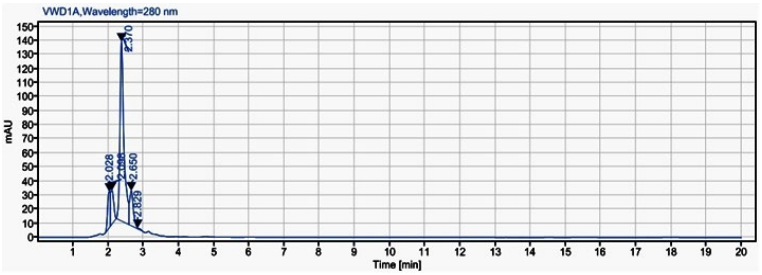

Fig. 4. Carrot sample Chromatogram

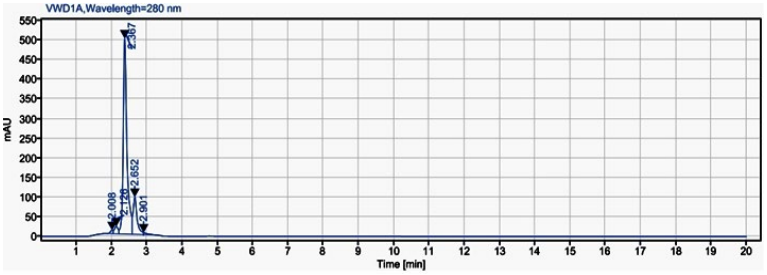

Fig. 5. Grape Sample chromatogram

Method validation

The method validation study was performed by the guidelines from ICH and Association of Official Analytical Chemists.Validation parameters like LOD and LOQ, Precision at LOQ level, System suitability, Specificity, Accuracy and Linearity were studied. The standard solution of 2.5 mL is poured into a volumetric flask of 10 mL and filled it up to the mark with methanol for determining LOQ and for LOD determination 3.3 mL solution into 10 mL volumetric flasks of 10 mL and dilute with methanol to the volume. The areas of LOQ and LOD standards were presented in Table 2 and six replicates were injected to study the system suitability. In the given Table 3, %RSD value of the area (peak) of all analytes were obtained less than 2.0%.

Table 2: Standard solution of LOQ and LOD(Area)

Name	Standard solution								
	First	Second	Third	Fourth	Fifth	Sixth	Average	SD	RSD%
Gallic acid	232.72	232.68	232.85	233.48	233.34	240.8	234.31	3.20	1.36
	Area of LOQ solution								
	First	Second	Third	Fourth	Fifth	Sixth	Average	SD	RSD%
Gallic acid	74.77	73.96	74.62	74.62	74.30	73.77	74.34	0.40	0.54
	Area of LOD solutions						Average	SD	RSD%
	First	Second	Third						
Gallic acid	20.99	20.96	20.98				20.98	0.02	0.07

RSD: relative standard deviation SD: Standard deviation

All analytes' area (peak) %RSD values were less than 2.0 percent, as shown in Table 3.

Table 3: Percentage of RSD

Name	Standard solution								
	First	Second	Third	Fourth	Fifth	Sixth	Average	SD	RSD%
Gallic acid	242.05	241.53	239.67	239.65	237.09	238.43	239.74	1.86	0.78

Specificity

Specificity is an important validation parameter and it discloses that the strategy is capable for tenacity of the analyte(s).

Procedure: 10 mg of GA standard was accurately weighed and diluted to the 100 mL volume with diluent. 1.0 mL of prepared solution was transferred into a volumetric flask of 50.0 mL and Sonicate to dissolve 1.0 mL of the prepared solution after being added approximately 5 mL of diluent and diluted to the volume with diluent in a 10.0 mL volumetric flask, labeled as stock Standard. Further 1.0 mL of this solution was taken into 20.0 mL volumetric flask and diluted until the desired volume was reached. The volume with diluent, labeled as standard solution.

Linearity

A linear response was observed from mode of detection and with reference to concentrations over the range of concentrations of the standard material(10ppm) uncovers linearity by planning in the reach 25%-200% concentration of Impurities. Injected all five dilutions of linearity solution (25%,50%,100%,150% and 200%) followed by blank. Record the peak area of product. Tables 4 provided linearity areas, and Fig. 6 displays a linearity graph.

Accuracy

To demonstrate the accuracy for GA impurities, recovery is performed by taking solutions(in the 50 percent, 100 percent, and 200 percent) of the proposed active concentration final product. The GA mean value was calculated and reported after each level was performed in triplicate. The LOQ level, RSD percentage, was less than 15%. In this parameter, the acceptance criteria for GA impurities recovery rates are between 80.0% and 120.0% for each concentration level. Accuracy and recovery results were and shown in Table 5 & 6.

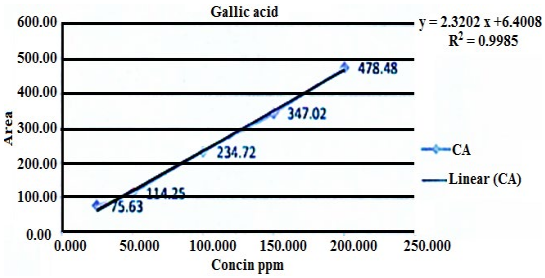

Fig. 6. Linearity graph

Table 4: Linearity

S. No	Injection Id	Areas of linearity	
		Percentage	Gallic acid
1	S1	25	75.63
2	S2	50	114.25
3	S3	100	234.72
4	S4	150	347.02
5	S5	200	478.48
			Correlation coefficient: 0.9985

Table 5: Accuracy

LOQ					
First	Second	Third	Average	SD	%RSD
69.94	73.62	77.84	73.80	3.95	5.36
		Accuracy at 50%			
115.28	119.05	120.89	118.41	2.86	2.42
		Accuracy at 100%			
241.18	241.51	234.51	239.07	3.95	1.65
		Accuracy at 200%			
453.25	484.76	475.37	471.13	16.18	3.43

Table 6: Recovery percentage

Name	LOQ Result	Accuracy at 50% Result	100% Result	200% Result
Gallic acid	107.84	103.21	107.03	100.41

CONCLUSION

The GA substance in fruits and vegetables varies according to cultivators, plant parts, and geographical regions.The GA substance in fruits and vegetables was studied by conventional extraction using methanol as solvent and later by using HPLC technique. Chromatographic conditions such as

C18 column (250mmx4.6mm, 5µm), detection at 280nm, flow rate 1mL/min etc were applied for the determination of GA. The LOD and LOQ parameters were in the ranges of 0.11–0.9 and 0.9–3.0 µg/mL, respectively. The reported method had advantages like simple, fast, and accurate.

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