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NOTE

A New Spectrophotometric Method for the Assay of Plant Phenolics

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A new spectrophotometric method for the assay of total phenolics in plants is reported. Neutral ferric chloride solution has been used as a complexing agent. This is a simpler method than the earlier reported ones for the phenolics assay.

Key Words: Total phenolics, Spectrophotometric assay.

Phenolics are an important constituent of plants and have significant role. Among the polyphenolics, flavonoids are found to be active as therapeutic agents. They possess anti-inflammatory, hypoglycemic, anti-tumour, analgesic, anti-microbial, anti-parasitic, anti-ulcer and anti-oedamatic activities[1–3]. Recent studies have indicated that flavonoids prevent the accumulation of cholesterol in blood vessels[4].

Assay of total phenolics is, therefore, an important aspect of phytochemistry as this establishes medicinal and economical importance of a particular species. Earlier method of total phenoics assay is that of Makkar *et al.*[5] which uses Folin-Ciocalteu reagent which is not so easy to prepare in laboratory. Another method, that was reported by Singh *et al.*[6], involves the use of $FeCl_3$-$K_3[Fe(CN)_6]$ in acidic medium. This method is not an authentic one as one gets a suspension rather than a clear solution when the above reagent is added to the plant extract.

Hence, the search of a new technique for the total phenolics assay became imperative. In this communication, spectrophotometric estimation of total phenolics (as gallic acid equivalent) using neutral $FeCl_3$ solution[7] is reported which is very simple and authentic at moderate concentrations of phenolics.

All spectrophotometric readings were made on Systronics UV-Visible spectrophotometer 119.

25 mg of gallic acid was dissolved in 5 mL of ethanol. From this ethanolic solution, 1 mL each was pipetted out in four different test tubes and each was diluted 20, 30, 40 and 50 times. From each sample, 1 mL was again pipetted out, 3 mL of neutral $FeCl_3$ added followed by the addition of 3 mL of water. Absorbances of these solutions were observed at 296 nm (Table-1) since Gallic acid-neutral $FeCl_3$ complex has λ_{max} at 296 nm.

TABLE-1
ABSORBANCE OF GALLIC ACID SOLUTIONS OF DIFFERENT CONCENTRATIONS

Concentration of the gallic acid solution (mg/mL)	Absorbance
0.100	1.200
0.125	1.360
0.166	1.660
0.250	2.106

Absorbance against concentration of gallic acid solutions was plotted. A straight line curve was obtained (Fig. 1).

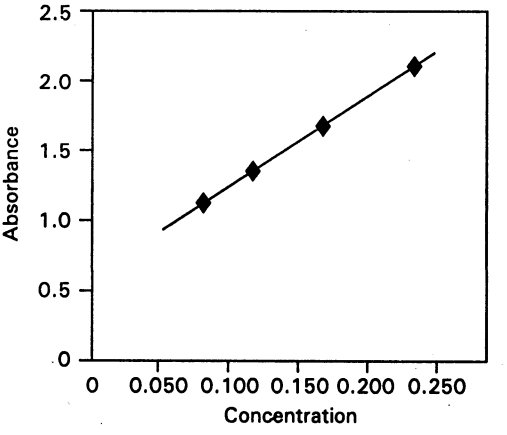

Fig. 1. Plot of absorbance *vs.* concentration of gallic acid solution (in mg/mL)

However, at higher and lower concentrations, straight line curve was not obtained as expected since Beer's law is followed at moderate concentrations only.

Straight line curve on plotting absorbance against concentration shows that Beer's law is followed. Hence, concentration of phenolics (gallic acid equivalent) in unknown solutions can be determined. This method can, therefore, be used to determine amount of phenolics in plant parts. However, for very low and very high concentrations of phenolics, spectrophotometric method is not completely reliable.

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