



## Original Article

# Isolation of Caffeine and Spectrophotometric Determination of Tannins in Black, Green and Grey Tea Bags

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A rapid, and accurate direct spectrophotometric method was developed for the spectrophotometric determination of tannins in tea samples after isolating caffeine. This method is based on formation of yellowish green coloured chelate complex between tannins and Cu(II) in alkaline medium. Copper ions are most effectively chelated by the constituents of tannic acid. The optimum conditions for the determination of tannins were established. Absorbance measurements were made at 420 nm and the calibration graph was linear from 10-80 micrograms ml<sup>-1</sup> of tannic acid. The molar absorptivity was found to be  $4.3 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ . The regression coefficient was found to be 0.994. The chelate in the extract was found to be stable for 3-4 hours. Effect of reagent concentration was studied. The Relative Standard Deviation for the determination of tannins in a tea sample containing 3.00% of tannins was found to be with 2.98%. Most of the ingredients commonly found in tea samples do not interfere with the determination. Different tea samples black, green and grey were analysed for their tannin content using the proposed method. The results obtained with the proposed method showed good agreement with those calculated by using known reference method.

**Key words:** Tannic acid, Copper sulphate, Teabags, Spectrophotometer.

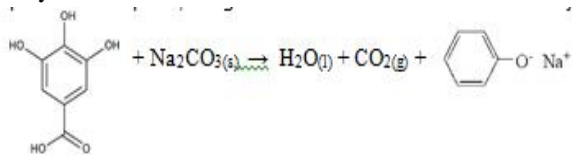
## 1. INTRODUCTION

Caffeine, an alkaloid stimulant is similar to the purine structures of DNA, affect biochemical pathways in the body<sup>1</sup>. The proper extraction process helps to isolate the caffeine within a tea bag to yield a pure solid. The mass of this solid obtained gives the actual yield of caffeine in the tea. Caffeine can be mixed with the methylene chloride, solvent that is both volatile and insoluble to water. Caffeine has a greater affinity for methylene chloride and will easily dissolve in this solvent over water. Along with caffeine, tea bags also contain organic substances called tannins<sup>1</sup>. Both

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caffeine and tannic acid are capable of dissolving in water. Greater polarity of caffeine induces dipole–dipole interaction and hence caffeine has more affinity towards water . By using sodium carbonate, tannic acid could revert back into phenol salt, a polar, inorganic molecule that is insoluble in methylene chloride.



Tannins are secondary plant metabolites with different biological activities and are found in many feeds of plant origin. Tannins are present in vegetal products as complex mixtures in which predominate esters of some polyphenolic acids with glucids, in the case of hydrolysable tannins, or products of catechin condensation, able to give steady, characteristic bounds, with the amino acids from proteins structure<sup>2-5</sup>.

Polyphenols have been considered previously to be non-nutrients, or even to be toxins, as some tannins have toxic effects such as binding to proteins, reducing the absorption of proteins, minerals and some vitamins, suppressing bodyweight gain, and inducing hepatic necrosis and methohemoglobinemia.<sup>6-11</sup> However, polyphenols have been recently recognized as functionally active molecules, possessing antioxidant, anticancer, antimutagenic properties, as well as exerting protective effects against cardiovascular and other diseases.<sup>12-21</sup>

In addition, polyphenols have been shown to have numerous health protective benefits, including lowering blood lipids and blood sugar, enhancing blood circulation, and blocking the action of carcinogens, which together contribute to the antiaging effect.

The main objective of the present study was to optimise a simple and rapid spectrophotometric method for the determination of tannins . In order to validate the method the results were tested with Reference method<sup>22-23</sup>

## 2. MATERIALS AND METHODS

**Samples:** Different weight of tea samples of black tea, green tea and grey tea were boiled into hot water for about 30 minutes hot solution was allowed to cool and solution was filtered .The residue was washed and checked for complete removal of tannins.

### 1000 ppm solution of tannic acid:

A fresh stock solution of tannic acid was prepared by dissolving 0.1 g of tannic acid in 100 ml of distilled water. The working solution were prepared by the appropriate dilution of the stock standard solution.

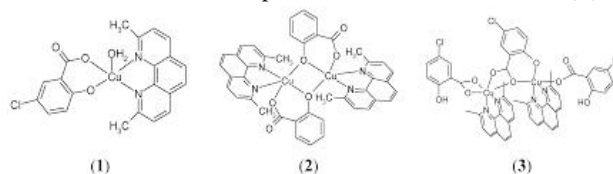
**0.1% of copper sulphate solution:** The reagent was prepared by dissolving 0.1g of A.R Copper sulphate in 100 ml distilled water.

**Equipments and Instruments:** All the glasswares used for the experimental purpose were made up of pyrex or Borosil

glass. The burette, pipette and standard flasks were calibrated by the method described by vogel. The absorption measurements were carried out on a spectrophotometer, model EQ-822, supplied by Equiptronics. The spectrophotometer was calibrated by measuring the absorption spectra of potassium chromate in potassium hydroxide solution and that of potassium permanganate in sulphuric acid solution.

### Recommended Procedure: Procedure for spectrophotometric determination of tannic acid:

To different aliquots containing 10-80 microgram / ml of standard solution of aqueous tannic acid, 2 cm<sup>3</sup> of 0.1% Cu(II) solution was added, followed by addition of 1ml of 0.05 ml KOH .The final volume was made to 50 cm<sup>3</sup> with distilled water. The amount of tannic acid was determined from a calibration curve prepared by processing solutions containing known amounts of tannic acid through recommended procedure and plotting the graph of absorbance against the concentration of tannic acid in ppm Structures of chelate complexes of tannic acid with Cu(II)



### Reference method:

A spectrophotometric method based on the principle that tannin forms coloured complex with phosphotungstomolybdic acid was applied to estimate the tannin content in tea samples. A standard solution of tannic acid of strength 1mg/ml was prepared. 10ml of this solution was made upto 100ml to give 100 ug / ml tannic acid .Linearity of tannic acid was studied in the concentration range of 3 to 30µg/ml. To each flask containing the requisite concentration; Folin-Denis reagent (0.5 ml), sodium carbonate solution (1 ml) and distilled water (up to 10 ml) was added. The absorbance was read at 760 nm within 30 mins of the reaction, against the reagent blank. A triplicate analysis was carried out.

### Isolation of caffeine and analysis of tannins in black, Green and Grey tea bags:

2.00 gm, 1.804 gm and 1.921 gm of tea samples of black tea, green tea and grey tea were boiled into hot water for about 30 minutes. The hot solution was allowed to cool and the caffeine was then extracted from the water with dichloromethane, which is an organic solvent that is insoluble in water. The solubility of caffeine is more in dichloromethane (140 mg/ml) than it is in water (22 mg/ml), so it dissolves to maximum extent in dichloromethane. However, the tannins are slightly soluble in the dichloromethane. But the caffeine was separated from the tannins by reaction with sodium carbonate to the water and the tannins were converted to phenolic anions, which are not soluble in the dichloromethane but are soluble in highly

polar water. Aqueous layer obtained after isolation of caffeine was diluted to 250 ml .1ml of above diluted solution was used for the analysis of tannins with the proposed method.

Validation parameters: Different parameters like accuracy ,precision, Linearity and range were considered to validate the developed method.

### 3. RESULTS AND DISCUSSION

#### Absorption spectra:

The proposed method involved formation of yellowish green colored chelate complex between tannic acid and Cu (II) ions in a alkaline medium. The absorption spectra of chelate against the reagent blank is shown in figure, whereas absorbance of Cu(II) in alkaline medium against distilled water was negligible at all wavelengths. The figure revealed that the complex has maximum absorbance at 420 nm. The reagent has not appreciable absorbance at specified wavelength. The color development was instant and the complex was found to be stable for four hours.

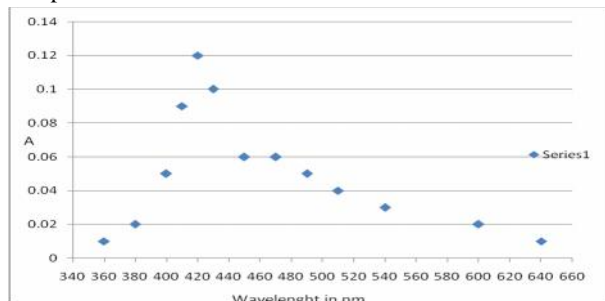


Fig 1: Absorption Spectra( Series1-complex vs blank)

#### Calibration curve:

A stock solution of tannic acid (1000 ppm) was used for the preparation of working solutions .Working solutions of TA were prepared immediately before use in order to prevent loss of analyte due to its instability at low concentrations.

To different aliquots containing 10- 80 micrograms/ ml of standard solution of aqueous tannic acid, 2 cm<sup>3</sup> of 0.1% Cu (II)solution was added, followed by addition of 1ml of 0.05 ml KOH .The final volume was made to 50 cm<sup>3</sup> with distilled water. The measurements were carried out at 420 nm.

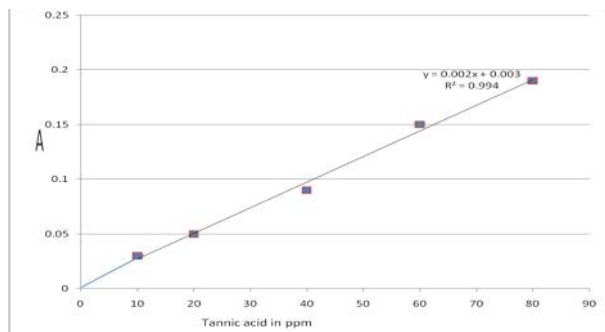


Fig2. Calibration curve

Parameter	Values
Absorption maxima	420 nm
Beer's range	10-80 ppm
Regression equation	Y=0.002X +0.003
Co- correlation coefficient	0.994

Table 1: Isolation of caffeine:

Sample	Weight of caffeine(%) w/w) obtained	
1.Black tea (Tajmahal, Brooke bond) -	0.103 gm	5.15%
2.Green tea(Green tea and apple, Twinings )	0.033 gm	1.83%
3.Grey tea:(Twinings )	0.023 gm	1.20%

#### Quantitative Estimation of Tannins in Tea samples:

The content of tannins in three tea bags, Black tea, Green tea, and Grey tea (expressed as tannic acid) was determined by the proposed method and the results were checked by the Folin-Denis method.<sup>23</sup> The results show that there is good agreement between the proposed and reference method, indicating the accuracy of the former.

#### Accuracy and Precision:

The precision and accuracy of the developed method for the determination of tannins was tested by analyzing the solutions containing 80 micrograms /ml of tannins. The regression coefficient was found to be 0.994.

The Relative Standard Deviation for the determination of tannins in a tea sample containing 3.00% of tannins was found to be with 2.98 %.

Table 2: Estimation of tannins

Samples of Tea	Contents of tannins (%) #		Relative Standard deviation from reference method (%)
	Proposed method@	Reference method Folin -Denis method	
Black Tea ( Taj mahal, Brooke bond)	4.13		
	4.084.13(1.3)	4.63	3.4
	4.09		
Green Tea(Green tea and apple, Twinings )	3.19		
	3.13 3.12(2.2)	2.83	5.8
	3.05		
Grey Tea(Twinings)	3.2		
	3.15 3.21(2.4)	2.88	5.7
	3.3		

@Relative standard deviation (%) given in parentheses # expressed as percent tannic acid

### 4. CONCLUSION

The proposed spectrophotometric methods for the determination of tannins is simple, accurate .The reagents

used in the proposed method are readily available in the laboratories and the amount of reagent used is much less, whereas the Folin-Denis method requires 50 g of sodium tungstate and 20 g of phosphomolybdic acid per 500 ml of solution. The proposed method does not require heating, whereas reference method requires refluxing for 2 hours and preparation of sodium carbonate solution demands overnight cooling. The assay methods do not involve any stringent reaction conditions, and non-interference from associated substances in the tea samples. The methods developed have been utilized to determine tannins in various tea samples.

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