Extraction of Polyphenols from Dried Tea Leaves

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Abstract— Polyphenols, a group of compounds present in tea leaves as major constituent, is known for its antioxidant property. It was extracted from dried and ground tea leaves. Enzymes in the fresh tea leaves were deactivated prior to drying followed by grinding and sieving. Caffeine, the other pharmacologically important compound, was also extracted as a byproduct. Crude extraction was made with water and subsequently the extract was concentrated for decaffeination with 1,2-Dichloromethane. Polyphenols were extracted from the decaffeinated crude extract with ethyl acetate. Ethyl acetate was removed from the extract to get dry solid polyphenols. Total polyphenols in the product was estimated by UV-Vis using Folin-Ciocalteu Phenol reagent, methods as described in ISO/DIS 14502-1. Catechins, the major component of tea polyphenols, were analysed by High Performance Liquid Chromatography (HPLC) for individual catechin present, as described ISO/CD 14502-2.

Index Terms— caffeine, catechin, extraction, polyphenols

1 INTRODUCTION

Tea is the most popular beverage in the world, only next to water. Its popularity is attributed to its sensory properties, relatively low retail price, stimulating effects and potential health benefits. Tea plant *Camellia Sinensis*, originally from Southeast China, gradually expanded to India, Sri Lanka and further into many tropical and sub-tropical countries. The most important chemicals present in tea, which are of considerable pharmacological significance, are the polyphenols and caffeine. Whatever the exact chemical identity, whole range of very similar-looking compounds with hydroxyl groups sticking out are ideally referred to as polyphenols. Some of the major constituent groups present in tea are briefly described below:

Polyphenols (Flavonoids): Green tea leaves contain many types of flavonoids, the most important of which are the flavanols (catechins), the flavonols and the flavanol glycosides. Tea catechins are water-soluble, colourless substances, which impart the bitter and astringent characteristic of green tea.

Caffeine and other Xanthines: Tea leaves contain 2.5 to 4.0% caffeine on a dry weight basis and much smaller quantities of the related methylxanthine theobromine. There are also lipids, amino acids, minerals, volatiles, enzymes are present in minor amounts. Average composition of a fresh tea flush of Assam Variety is as shown in table 1.

TABLE 1

Composition of Fresh Tea Flush: Assam Variety

Substances soluble in hot water	
Flavanols	17-30
Flavonols and flavonol glycosides	3-4
Leucoanthocynins	2-3
Polyphenolic acids and depsides	5
Total phenolics	25-35
Caffeine	3-4
Amino acids	4
Simple carbohydrates	4
Organic acids	0.5
Substances partially soluble in hot water	
Polysaccharides	13
Proteins	15
Ash	5
Substances insoluble in water	
Cellulose	7
Lignin	6
Lipids	3
Pigments	0.5
Volatile substances	0.01-0.02

Tea as a beverage is attractive for its aroma as well as the liquor that comes from the component present.

Table 2

Phenolic Compounds(Flavanols) In Tea Flush

Particulars	Short	Formula	MW	%Dry wt
FLAVANOLS				
(-)- Epicatechin	(-)-EC	$C_{15}H_{14}O_6$	290	1-2
(-)- Epicatechin gallate	(-)-ECG	$C_{18}H_{18}O_{10}$	442	3-6
(-)- Epigallocatechin	(-)-EGC	$C_{15}H_{14}O_7$	306	3-6
(-)-Epigallocatechin gallate	(-)-EGCG	$C_{22}H_{18}O_{11}$	458	9-12
(+)- Catechin	(+)-C	$C_{15}H_{14}O_6$	290	1-2
(+)- Gallocatechin	(+)-C	$C_{15}H_{14}O_7$	290	1-2
(+)- Gallocatechin gallate	(+)-GCG	$C_{22}H_{18}O_{11}$	458	
(+)- Catechin gallate	(+)-CG	$C_{18}H_{18}O_{10}$	442	

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Total Polyphenols	25-35

2 EXPERIMENTAL PROCEDURE

2.1 Extraction of Polyphenols from Dried Tea Leaves

To crudely crushed dried tea leaves(30 g) hot water (60^0 C) is added in the ratio 1:20(with periodical stirring to deactivate enzymes). The boiling mixture's filtrate is collected(three times). Rotavapour(water bath at 60^0C) is used to concentrate the tea solution. The volume of the concentrate is 245 ml.

2.2 Decaffeination

To the concentrate an equal volume (245 ml) of CH_2Cl_2 is added and shaken. The lower part being caffeine (chlorophyll, lipid, carbohydrate etc) dissolved in CH_2Cl_2 and upper part being the undissolved remaining concentrate (specific gravity of CH_2Cl_2 > specific gravity of H_2O). This liquid-liquid extraction is repeated six times. The volumes of the extracted mixture of caffeine, carbohydrate, lipid, chlorophyll and CH_2Cl_2 obtained each time are 195 ml, 240 ml, 210 ml, 250 ml, 240 ml and 250 ml respectively. The solution so obtained now is concentrated in a rotavapour without vacuum . The CH_2Cl_2 is recovered in the matter plastered inside the round bottom flask. The weight of the caffeine mixture is 0.93 g. Water is added to the dried caffeine mixture and then filtered. The residue comprises of chlorophyll . Filtrate containing the rest of the mixture has a volume of 42 ml. The filtrate is now concentrated.

2.3 Refining of Crude Caffeine

To the crude caffeine hot distilled water is added in the ratio 1:20. Its pH is 5.6(approx.). The solution is heated in a water bath(80^0C) with constant stirring for half an hour. A sticky layer (trace amount of chlorophyll that persists even after filtration)is removed from the surface using the stirrer. Again the pH is 5.6 approximately. The solution after evaporation is now filtered; residue being chlorophyll is now removed. A few drops of concentrated HCl is added to the filtrate and heated to 70^0-80^0C in the water bath and stirred to bring the pH of the filtrate down to 3 approximately because at a pH of 3, protein (mainly tannin) present in the filtrate precipitates out. Filtration is done and caffeine is obtained. To the filtrate CaO is added to bring the pH from 3 to the initial pH of 5.6 .Now charcoal is added to the solution to decolorize it and the temperature is raised to 80^0-90^0 C in the water bath with periodic stirring. The pH is 5.2(approx.) now. The solution is filtered to decolorize and then heated to get a concentrated solution. The solution is allowed to cool, forming a white lumpy mass, which is vacuum filtered and oven dried, finally giving pure white caffeine crystals.

2.4 Extraction of Polyphenols from Decaffeinated Crude Extract

The undissolved remaining tea concentrate is mixed with 250ml ethyl acetate and 0.1 gm ascorbic acid (to prevent oxition) in a separating funnel. The mixture is immissible, the upper yellow part is polyphenol dissolved in ethyl acetate and the lower part is the remaining tea solution (oil, fats, lipids, etc). These liquid- liquid extraction (without the addition of ascorbic acid) is done five more times. The volume of the extracted mixture of ethyl acetate and polyphenols obtain each time are 250ml, 240ml, 255ml, 270ml, 255ml, 255ml respectively.The solution is then concentrated and the polyphenols is dried and stored in desicator to prevent stickiness. Exposure to air decomposes the polyphenols. The weight of the polyphenol concentrate is 5.8 gm.

The figure below shows the brief flow chart of the process:

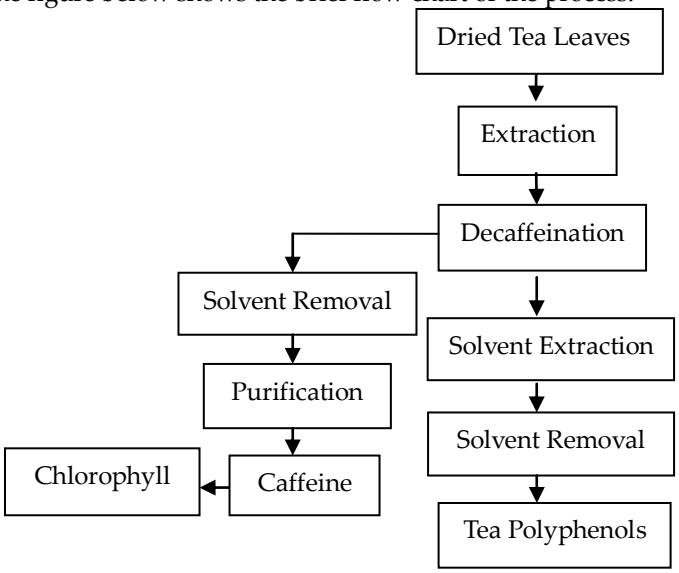


Fig. 1. Flow Chart for the Extraction of Polyphenols from Dried Tea Leaves

3 ANALYSIS OF PRODUCT COMPOSITION

3.1 Estimation of Total Polyphenols By UV-Vis

To 0.5 gm of extracted polyphenols 5ml of acetonitrile is added and water is added to 50ml mark of the volumetric flask. The solution is diluted 100 times with water.The solution is made to react with dilute Folin-Ciocalteau phenol reagent(FC) and sodium carbonate (Na_2CO_3) which is then compared with a blank solution of Folin-Ciocalteau reagent and sodium carbonate. Now considering the blank solution as A and the polyphenol solution as B, the reagent added to these in ml are-

Table 3
Preparation of solutions A and B

The greenish blue colour of the solution B indicates the presence of polyphenol in the solution. A sample of the solution B is further tested in the UV Spectrophotometer which indicates the optical density of the substance which is used for the calculation of total polyphenol content.

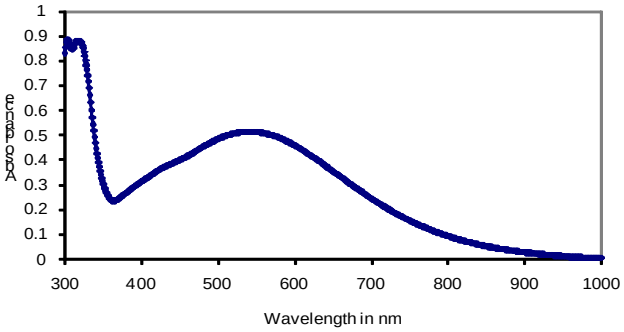


Fig. 2. UV-Vis spectra of Total Polyphenols in the Product

The standard Gallic Acid calibration curve is shown below:

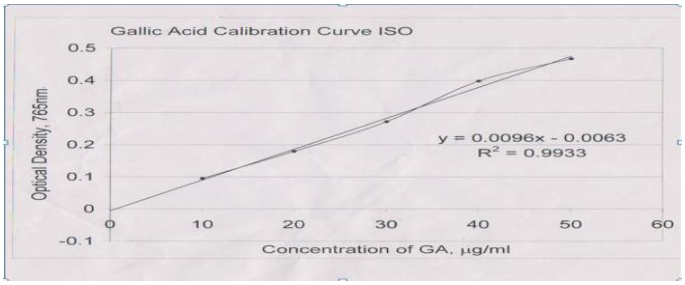


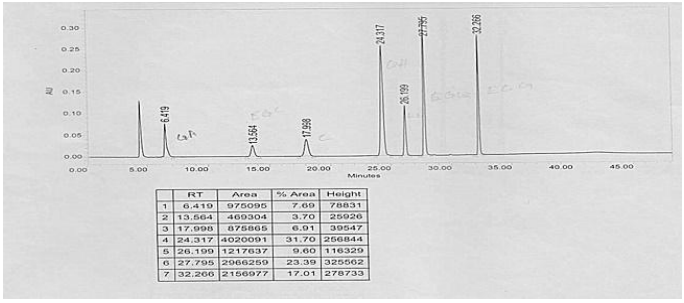
Fig. 3. Standard Gallic Acid Calibration Curve for Estimation of Total Polyphenols

The total polyphenol content expressed as a percentage by mass on a sample dry matter basis is given by the formula (as per ISO/ DIS 14502-1):

$$\frac{(ODs - ODi) \times SV \times DF \times 100}{Slope_{std} \times SM \times 10000 \times DMC} \quad (1)$$

Where,
ODs=Optical Density(sample)
ODi=Optical Density(intercept)
SV=Sample Volume
DF=Dilution Factor
SM=Sample Mass
DMC=Dry Matter Content

The HPLC curve for standard and sample catechin mixtures are shown in figures 5 and 6:



A	B
1 mL H_2O+5 mL FC+4 mL Na_2CO_3 (Na_2CO_3 is added to the solution after 3-5 minutes of adding FC)	1 mL of the solution made in step (18) + 5 mL FC + 4 mL Na_2CO_3 (Na_2CO_3 is added to the solution after 3-5 minutes of adding FC)

Fig. 4. HPLC Curve for Standard Catechin Mixture

And the HPLC curve for the sample mixture is shown below:

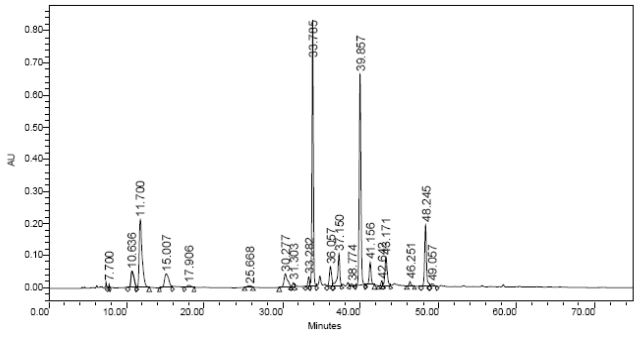
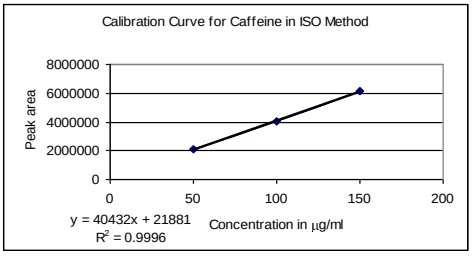
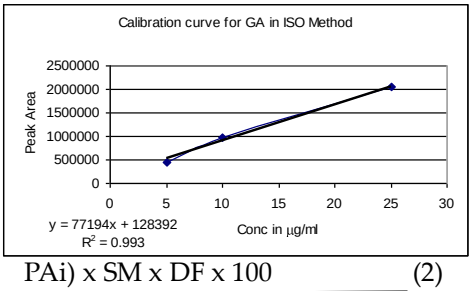


Fig. 5. HPLC Chromatogram of Product (Polyphenols)

The individual component content, expressed as a percentage by mass on a sample dry matter basis, is given by the formula (as per ISO/CD 14502-2):





$$\frac{(PAs - PAi) \times SM \times DF \times 100}{Slope_{std} \times sm \times 10000 \times DMC} \quad (2)$$

Where,
PAs=Peak Area(sample)
PAi=Peak Area(intercept)

SV=Sample Volume
DF=Dilution Factor
SM=Sample Mass
DMC=Dry Matter Content

The figures below show the calibration curves for caffeine, gallic acid and the individual component (five catechins) found in the analysed polyphenols.

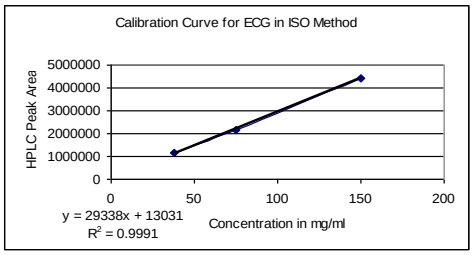
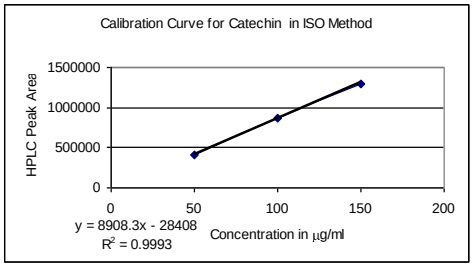
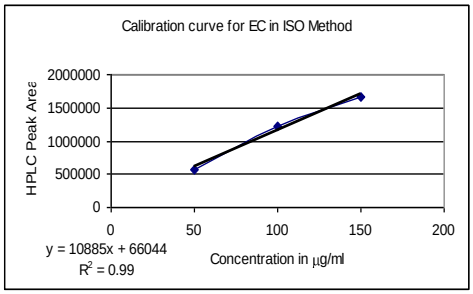
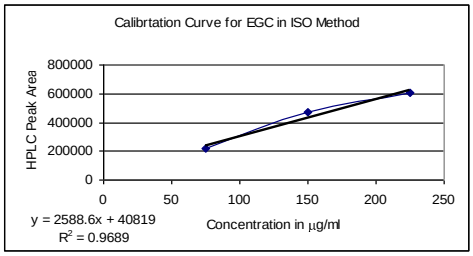
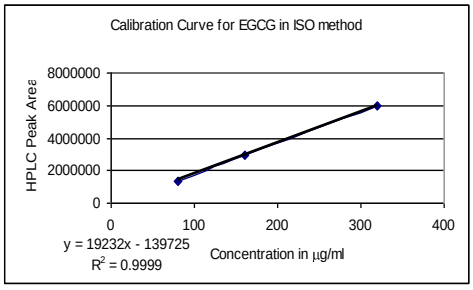


Fig. 6. Calibration Curves for five Catechins, Caffeine & Gallic Acid by ISO method









3.2 HPLC Analysis of Polyphenols

0.5 gm of the extracted polyphenol is taken in a 50ml volumetric flask and 5ml HPLC grade acetonitrile is added to it. It is then diluted with water upto the mark. Weigh to the nearest 0.01 g, 0.5 g of EDTA (Ethylenediamine tetraacetic acid disodium salt, dehydrate) into a 50 mL one-mark volumetric flask. Add sufficient warm water to half fill the flask. Swirl to dissolve the EDTA, cool to room temperature, dilute to the mark with water and mix. Weigh, to the nearest 0.01 g, 0.5 g of L-ascorbic acid into a 50 mL one-mark volumetric flask. Dissolve in water, dilute to the mark and mix. Using a pipette transfer 5 mL of EDTA solution, 5 mL ascorbic acid solution and 10 mL acetonitrile into a 100 mL one-mark volumetric flask. Dilute to the mark with water and mix.1ml of solution of Polyphenol + Folin- Ciocalteu Phenol Reagent + Sodium Carbonate, 4 mL of the stabilizing solution is then mixed in a volumetric flask and taken for HPLC test. The HPLC test indicates the percentage of the constituents of polyphenol. The residue containing starch is taken on a petridish and then concentrated in a hot water bath.
Weight of residue is 6.14 g.

4 RESULTS AND DISCUSSION

4.1 Total Polyphenol Content

1. Total refined caffeine content = 2.56%

The dried tea leaves have undergone decaffeination using liquid-liquid extraction and the crude caffeine obtained is further refined through certain physical and chemical changes.

2. Residue after evaporation = 20.47%

It is a by-product which is obtained while extracting polyphenols using ethyl acetate as solvent.

3. Concentrated polyphenols obtained after extraction = 19.33%

The polyphenol percent is confirmed through UV Analysis using spectrophotometer. The graph is shown in figure 3.
The optical density of the sample analysed is found to be 0.7967. This value is used for calculating the total polyphenol content in the extracted mass.
We compare the optical density value obtained with the stan-

dard Gallic Acid calibration curve as shown in figure 3.
The total polyphenol content expressed as a percentage by mass on a sample dry matter basis given by (1) (as per ISO/ DIS 14502-1) is *88.9849%*

4.2 HPLC Analysis As Per ISO/CD 14502-2

The catechins present in the mixture are identified by comparing with the HPLC curve obtained for standard catechin mixture (Fig. 4.).
The individual component content, expressed as a percentage by mass on a sample dry matter basis is given by (2) (as per ISO/CD 14502-2).
Comparing with the standard calibration curves for individual component, as shown in figure 6 and using the relation above we obtain the individual component content, expressed as a percentage by mass on a sample dry matter basis, as shown in the table below:

TABLE 4
Percentage Concentration of the Individual Components in Polyphenols

Component	Concentration (%)
Gallic acid	0.2819
Catechin	0.97
Epigallo catechin	0.0022
Caffeine	0.0314
Epicatechin	0.06327
Epigallo catechin gallate	5.0996
Epicatechin gallate	1.0266

5 CONCLUSION

Polyphenols and caffeine are successfully extracted from dried tea leaves by the method of solvent extraction. This method is found to be dependent on parameters like type of solvent, temperature, pH of the solution, solid-liquid ratio, particle size etc. The number of extraction stages depends on the efficiency of the equipment used. It is the method that is being employed industrially for large scale production today. Another method called membrane separation is being studied by tea researchers in the field of extraction of polyphenols. Though it has shown promise in the production of polyphenols of better quality (than that of solvent extraction) however the same cannot be said in the quantity of the product. It is still in the pre-employed, presently research stage

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