Extraction and Estimation of Different Active Constituents present in the different brands of Green Tea by using Analytical Methods

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Abstract. Green tea (camellia sinensis. L) is one of the most popular highly used in nutritional supplement in the world. Ethanol and water were used as a solvent to extract active constituents from different brand of green tea like Lipton, Tetley, and Ecovalley natural green tea. The active constituents of green tea can be determined by analytical instruments like IR spectrophotometer used to know functional groups and TLC technique is used to know purity and no of different constituents of different brands of green tea. The absorbances can be determined by Colorimetry and UV spectroscopy. The DSC can be used to know the melting point of different constituents of different brands of green tea. The antioxidants present in the different brands of green tea estimated by using DPPH method. Predict the Immune booster activity of (-) epigalocatechin gallate (EGCG) with target protein 3NG5 by docking study using the software swiss docking. By the above analytical methods, Ethanolic extract shows high percentage of yield than hot water extraction. Lipton green tea brand shows more active constituents compared to other brands of green tea.

Keywords: Green tea, Antioxidant activity, DPPH, Docking study.

1. Introduction
Tea is one of the most popular drinks, obtained by water infusion of tea leaves (Camellia sinensis)1. Green tea leaves are grown worldwide at about 22 percent (640,000) tons per year. Nowadays, tea is manufactured all over the world in thirty countries. A wide variety of different green tea brands are sold on the world market2. Green tea contains polyphenols, vitamins, proteins, organic acids, sugars, and glycosides, among others. The content of flavanols and flavanones in green tea are very compared to those in other foods3. The polyphenols tea leaves include catechins, and phenolic acids have been considered the main players in these beneficial effects on the human health. The major tea catechins are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC). Tea contains around 5-7 percent of the minerals that play a significant function in the human body, primarily potassium, copper, iron and manganese.5
The different brands of green tea available in India. These are Lipton, Tetley, Eco valley natural green tea etc... Lipton is a British brand of tea, owned by Unilever. The company is named after its founder Thomas Lipton. Tetley is a beverage manufacturer founded in 1837 in Yorkshire, England. Eco valley sources organic green tea leaves from Nilgiris hills, which is 8000 ft from the ground. Active constituents of these three products can be estimated by using analytical methods like colorimetry method, TLC, UV spectroscopy, IR spectroscopy, Differential scanning colorimeter.

2. Materials and Methods
The three brands of green tea used for project work purchased from market, the chemicals and solvents were procured from the lab of IPT, SPMVV, Tirupati.

2.1. Extraction methods

2.1.1 Ethanolic extraction of green tea constituents. Approximately weighed about 1.4 g of green tea in a 250ml round bottom flask(RBF) and apply about 100ml of ethanol to the flask. Reflux for 60 minutes at boiling temperature of around 78 °C. The temperature is continuously controlled by the use of the water bath. After the extraction process compounds are dried under sun shade, the dried compound was weighed. The percent yield was found to be 8%. Three brands of extractions are taken using the above process.

2.1.2 Hot water extraction of green tea constituents
Approximately weigh about 1.4 g of green tea into a 250ml RBF, to it add water about 100ml into flask Carry the extraction for 20minutes at the boiling temperature of 100 °C. Temperature is maintained constantly. After the dried compound was weighed. The % yield was found to be 3.5 %. By the above method three brands of extraction is taken.

2.2 TLC of green tea constituents
TLC of green tea ethanolic extracted constituents and water extracted constituents are carried by preparative plates. The dried constituents were dissolved in methanol and solvent system was chloroform: methanol as a mobile phase in the ratio 8:2. 

Figure 1. Structures of the different catechins
2.3. Chemical tests for testing of phenolic constituents present in the sample

2.3.1. Ferric chloride test: The sample is dissolved in 2 ml of ethanol and apply a few drops of dilute ferric chloride (FeCl₃) solution, which has transformed to purple after a few seconds. The colour indicates presence of phenols.

2.3.2. Shinoda test: Transfer small amounts of green tea in the conical flask and apply ethanol for ethanol extract preparation. After applying ethanol to the conical flask, shake the flask. Filter the solvent. Take approximately 2-3 ml of ethanol extract in the test tube and add 2 drops of conc. HCl, then add 2 pieces of magnesium ribbon to the test tube. Finally, it changes to the pink colour. It indicates the presence of flavonoids present in green tea.

2.4. Colorimetric Method
We have taken different concentrations of green tea constituents and observed the absorbance at different wavelength. From that λmax is observed and at the specific concentration showed specific λmax at 340nm. At 340 nm prepared the standard graph for ethanolic green tea constituents

2.5. UV-Spectroscopic Method
We have taken different concentrations of green tea constituents and observed the absorbance at different wavelength and λmax was found to be at 221.80nm.

2.6. IR method
Take approximately about 10 mg sample and add powdered potassium bromide about 100 mg into motor and triturate into fine mixture with the help of pestle. Under very high pressure (25000p sig) this finely ground mixture is pressed using minipress to form a small pellet (about 1-2mm thick and 1 cm in diameter) then place pellet in path of the beam of IR spectrometer using blank KBr pellet of identical thickness as reference beam.

2.7. Differential scanning calorimetry (DSC)
Sample preparation was done by Accurately weighed the sample about 10mg was taken and Spread them uniformly around the bottom of the pan to eliminate thermal gradients when using powde red or granular samples. Choose the side of your sample with the flattest surface for solid samples for a touch. Ensure that the pan bottom is smooth after encapsulating the sample. Flatten it by pushing the pan bottom on a flat surface if it is not. There should be the same material and configuration. The temperature should be maintained between 100°C to 200°C. This procedure is done for both ethanolic and water extracts of green tea constituents by differential scanning colorimetry (DSC).

2.8. DPPH method
In this work different types of green tea were evaluated, such as natural green tea brands like Lipton, Tetley, and Ecovalley. By adding 0.2 g of greentea extract to 10ml of distilled water, various tea samples were prepared and steeped for 5 minutes at 95-100°C. Using Whatman’s filter paper, the hot water samples were filtered and the filtrate was used for further examinations.

2.9. Docking study
Docking of protein and ligand: Docked the ligand with a protein by a swiss dock (www.Swissdock.In) which is a free online software. Flexibility was allowed for the side chains are 0A. By changing the clusters of a protein the binding site varies. Selected the binding site which has a less affinity to bind. Most favourable predicted binding models were found to be 2A₀ to the active site.
2.9.1. Details of protein structure for immune booster activity
Protein : 3NG5  
Organism: homo sapiens  
Chain: A and B  
resolution: 2.17Å  
Compound: (-) epigallocatechin gallate  
Full fitness: -1393 -35 Estimated (ΔG kcal/ mole): -7.96  
Bond length: 2.023Å  
Interactive amino acid and ligand: O4 SER

3. Results and discussion

3.1. Extraction constituents percentage yield calculation

3.1.1. Percentage yield of Ethanolic extraction of green tea constituent’s.  
The extraction process of Ethanolic green tea constituent’s prepared and the percentage yield is more in Lipton green tea constituents represented in Table 1.

Table 1. percentage yield of green tea constituents

| S. No. | Green tea brands                        | Percentage of yield |
|--------|----------------------------------------|---------------------|
| 1      | Lipton green tea                       | 8.3%                |
| 2      | Tetley green tea                       | 7.8%                |
| 3      | Eco valley natural green tea           | 7.0%                |

3.1.2. Percentage yield of Hot water extraction of green tea constituent’s.  
The Extraction process of hot water green tea constituent’s prepared and the percentage yield is more in Lipton green tea constituents in compare to other green tea brands in Table 2.

Table 2. Percentage yield of three brands of green tea constituents

| S. No. | Green tea brands                        | Percentage of yield |
|--------|----------------------------------------|---------------------|
| 1      | Lipton green tea                       | 7.2%                |
| 2      | Tetley green tea                       | 7.0%                |
| 3      | Eco valley natural green tea           | 6.6%                |

3.2. TLC of green tea constituents

Figure 2. TLC for Lipton green tea constituents both ethanolic and water extract  
Figure 3. TLC for Tetley green tea constituents both ethanolic and water extract  
Figure 4. TLC for Eco valley Natural green tea constituents both ethanolic and water extract
TLC for ethanolic extract of Lipton green tea constituents shows two spots where as in water extract one spot in Fig.2,TLC for ethanolic extract of Tetley green tea constituents shows one spot where as in water extract one spot in Fig.3,TLC for ethanolic extract of Tetley green tea constituents shows one spot where as in water extract one spot in Fig.4,Rf factor for ethanolic extract and water extract of Eco Valley natural green tea brands constituents shows more compared to other green tea brands in table 3.

### Table 3. Comparison of green tea brands by using TLC

| Green tea brands   | Ethanolic extract spots on TLC plate | Water extract spots on TLC plate | Rf values |
|-------------------|-------------------------------------|---------------------------------|-----------|
| Lipton            | Two spots                           | One spot                        | 0.6       | 0.5     |
| Tetley            | One spot                            | One spot                        | 0.66      | 0.55    |
| Eco valley natural| One spot                            | One spot                        | 0.7       | 0.62    |

3.3. **Chemical test**

### Table 4. Comparison of phenols present in the green tea constituents by ferric chloride test

| S. No. | Green tea brands       | Observation           |
|--------|------------------------|-----------------------|
| 1      | Lipton green tea       | Red color is observed |
| 2      | Tetley green tea       | Light red color is observed |
| 3      | Eco valley green tea   | Pale red color is observed |

### Table 5. Comparison of flavonoids present in the green tea constituents by Shinoda test

| S. No. | Green tea brands       | Observation           |
|--------|------------------------|-----------------------|
| 1      | Lipton green tea       | Pink color is observed |
| 2      | Tetley green tea       | Light pink color is observed |
| 3      | Eco valley green tea   | Pale pink color is observed |

Comparison of phenols present in the green tea constituents by ferric chloride test for Lipton green tea constituents shows red colour compared to other green tea brands in table 4 and 5. It indicates presence of phenols.
3.4 Colorimetric method

Table 6. Absorbances of green tea ethanolic extract at 340 nm by colorimetry

| Concentration | Maximum wavelength $\lambda_{\text{max}}$ | Absorbance |
|---------------|------------------------------------------|------------|
| 0.5ml         | 340nm                                    | 1.24       |
| 0.75ml        | 340nm                                    | 1.25       |
| 1ml           | 340nm                                    | 1.26       |
| 1.25ml        | 340nm                                    | 1.27       |
| 1.5ml         | 340nm                                    | 1.29       |

Table 7. Absorbances of green tea constituents water extract at 380 nm by colorimetry

| Concentration | Maximum wavelength $\lambda_{\text{max}}$ | Absorbance |
|---------------|------------------------------------------|------------|
| 0.5ml         | 380nm                                    | 1.18       |
| 0.75ml        | 380nm                                    | 1.19       |
| 1ml           | 380nm                                    | 1.21       |
| 1.25ml        | 380nm                                    | 1.23       |
| 1.5ml         | 380nm                                    | 1.25       |

Comparison of three brands of green tea constituents of both ethanolic and water extract at maximum wavelength by colorimetry in table 6 & 7. Ethanolic extract shows higher absorbance than water extraction. In this the constituents of Lipton green tea brand shows higher absorbance than other green tea brands.

Table 8. Comparison of three brands of green tea constituents of both ethanolic and water extract at maximum wavelength by colorimetry

| Green tea brands          | Colorimetric absorbances at $\lambda_{\text{max}}$ |
|---------------------------|-----------------------------------------------------|
|                           | Ethanol extract at $\lambda_{\text{max}}, 340$ nm | Water extract at $\lambda_{\text{max}}, 380$ nm |
| 1. Lipton green tea      | 1.12,1.14,1.18,1.22,1.25                         | 1.15,1.17,1.20,1.21,1.23                         |
| 2. Tetley green tea      | 1.06,1.07,1.08,1.11,1.13                         | 1.12,1.14,1.17,1.19,1.21                         |
| 3. Eco valley natural green tea | 0.99,1.01,1.05,1.07,1.09                  | 1.10,1.13,1.15,1.18,1.21                         |
3.5. **UV spectroscopic method**

**Table 9.** UV Absorances for ethanolic extract of green tea constituents at maximum wavelength

| Concentration | Maximum wavelength $\lambda_{\text{max}}$ | Absorbance |
|---------------|------------------------------------------|------------|
| 0.5ml         | 221nm                                    | 1.29       |
| 0.75ml        | 221nm                                    | 1.42       |
| 1ml           | 221nm                                    | 1.72       |
| 1.25ml        | 221nm                                    | 1.81       |
| 1.5ml         | 221nm                                    | 1.98       |

**Table 10.** UV Absorances for Hot water extract of green tea constituents at maximum wavelength

| Concentration | Maximum wavelength $\lambda_{\text{max}}$ | Absorbance |
|---------------|------------------------------------------|------------|
| 0.5ml         | 239nm                                    | 0.98       |
| 0.75ml        | 239nm                                    | 1.12       |
| 1ml           | 239nm                                    | 1.19       |
| 1.25ml        | 239nm                                    | 1.27       |
| 1.5ml         | 239nm                                    | 1.30       |

**Table 11.** Comparison of three brands of green tea constituents of both ethanolic and water extract at maximum wavelength

| Green tea brands | UV absorbances at $\lambda_{\text{max}}$. |
|------------------|-----------------------------------------|
|                  | Ethanol extract at $\lambda_{\text{max}}$, 221nm | Water extract at $\lambda_{\text{max}}$, 239nm |
| 1.Lipton green tea | 1.25,1.33,1.52,1.75,1.80                   | 0.95,0.99,1.05,1.12,1.20                  |
| 2.Tetley green tea | 1.20,1.33,1.54,1.76,1.83                   | 0.92,0.97,1.03,1.07,1.14                  |
| 3.Eco valley natural green tea | 1.20,1.34, 1.55,1.77,1.85,1.09            | 0.90,0.93,1.00,1.02,1.08                  |

Comparison of three brands of green tea constituents of both ethanolic and water extract at maximum wavelength by UV spectroscopy in Table 9&10. Ethanolic extract shows higher absorbance than water extraction. In this the constituents of Lipton green tea brand shows higher absorbance than other green tea brands.
3.6. The IR spectral data for three types of green tea brands

3365(O-H), 2923(C-H) str, 1697(C-H) bending, 1234(C-O) str, 1036(S=O) str

**Figure 5.** Lipton green tea constituents of ethanolic extract

3354(O-H) str, 2923(C-H) str, 1696(C-H) bending, 1236(C-O) str, 1036(S=O) str
Figure 6. Tetley green tea constituents of ethanolic extract

3360(O-H), 2924(C-H) str, 1695(C-H) bending, 1236(C-O) str, 1036(S=O) str

Figure 7. Eco Valley natural green tea constituents of ethanolic extract

3358(O-H), 2927(C-H) str, 1694(C-H) bending, 1238(C-O) str, 1036(S=O) str

Figure 8. Tetley green tea constituents of water extract
It is done for both ethanolic and hot water extraction of green constituents. Comparison of functional groups present in the both ethanolic and water extracts of green tea constituents by IR shows similar functional groups manly because of presence of flavonoids.

3.7. Differential scanning colorimetry

![Figure 9. DSC for ethanolic extract of Lipton green tea constituents](image)
Figure 10. DSC for ethanolic extract of Tetley green tea constituents

Figure 11. DSC for ethanolic extract Eco Valley natural green tea
Figure 12. DSC for ethanolic extract Tetley green tea

Comparison of both ethanolic and water extract of green tea constituents shows peaks by DSC. In this, Lipton green tea constituents shows four peaks, Tetley green tea constituents shows one peak and Eco valley natural green tea constituents shows three peaks it indicates Lipton green tea contain more active constituents than other brands of green tea.

3.8 DPPH radical scavenging activity

The DPPH free radical scavenging operation of various types of green tea was graded according to IC50 values: Eco valley natural green tea (29.93µg/ml) > Tetley (27.26 µg/ml) > Lipton (24,42 µg/ml) illustrated in Table 12. The lower the IC50 value, the greater the potential for antioxidants. It was observed that green tea from Lipton is significantly more successful in scavenging radical DPPH followed by natural green tea from Tetley and Eco valley.

Table 12. Average IC50(μg/ml) values of the green tea brands

| Samples                        | DPPH values |
|--------------------------------|-------------|
| Ascorbic acid(standard)         | 41.25±1.19  |
| Eco valley natural green tea    | 29.93± 3.56 |
| Tetley green tea               | 27.26± 3.12 |
| Lipton green tea               | 24.42± 2.26 |

Figure 13. Graphical representation of DPPH values of standard and green tea brands
1. Ascorbic acid, 2. Lipton, 3. Tetley, 4. Eco valley
3.9. Docking study of (-) epigallocatechin gallate present in green tea constituents shows Immune booster activity

![Docking structure of protein with ligand](image)

**Figure 14.** Docking structure of protein with ligand

- (-) epigallocatechin gallate strongly binds with ligand so it exhibits Immune booster activity.

4. Conclusion

Ethanolic extract shows more percentage yield than hot water extract, TLC shows a greater number of constituents present in Lipton green tea compared to other brands. By using colorimetry and UV spectroscopy Lipton shows maximum absorbance in specific wavelength, IR of ethanolic extract of green tea constituents and water extract of green tea constituents were observed and it indicates similar functional groups, DSC of both ethanolic extract of green tea constituents and water extract of green tea constituents was done and lipton graph having more peaks indicates more constituents. The melting point of ethanolic extract and water extraction of Lipton green tea shows more active constituents than other brands of green tea. The antioxidant activity in Lipton green tea is significantly more and having effective freeradical scavenging activity observed for lipton than other brands of green tea. The compound epigallocatechin gallate which having good binding property in docking study shows green tea also having immune booster activity. Lipton green tea brand contain more active constituents than other brands of green tea brands.

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