

Original Article

Isolation and Quantitative Evaluation of Caffeine from Different Commercial Tea Powders Available in Raigad District, Maharashtra

Asmita M. Patil

Caffeine Department of Chemistry Sheth. J. N Paliwala College, Pali- Sudhagad-Raigad, M.S

Manuscript ID:
BN-2026-030216

ISSN: 3065-7865

Volume 3

Issue 2

February 2026

Pp. 76-81

Submitted: 12 Jan 2026

Revised: 22 Jan 2026

Accepted: 10 Feb 2026

Published: 28 Feb 2026

DOI:

[10.5281/zenodo.19848310](https://doi.org/10.5281/zenodo.19848310)

DOI link:

<https://doi.org/10.5281/zenodo.19848310>



Quick Response Code:



Website: <https://bnir.us>



Abstract

Is a naturally occurring alkaloid widely found in beverages such as tea, coffee and cola, and is known for its central nervous system stimulant and mild diuretic properties. The amount of caffeine in tea varies depending on the brand, processing method and quality of tea leaves. The present study focuses on the extraction and comparative evaluation of caffeine from different commercial tea powder brands marketed in Raigad district, Maharashtra. Along with caffeine isolation, selected physicochemical parameters such as taste, pH, colour, bulk density and normality of aqueous extract were analysed to assess the quality of tea powders. Caffeine was isolated from tea samples using a liquid-liquid extraction method. A comparative study of different tea brands revealed variations in physicochemical properties and caffeine yields among samples. The findings highlight the importance of analytical evaluation of commercial tea products and provide useful information on caffeine content and quality assessment. This study demonstrates a simple and reliable laboratory method for caffeine extraction and characterization, which can be useful for academic research, quality control and further analytical investigations.

Keywords: Caffeine, tea powder, liquid-liquid extraction, chloroform extraction, sublimation, thin layer chromatography (TLC).

Introduction

Caffeine (C₈H₁₀N₄O₂) is a naturally occurring purine alkaloid commonly known as trimethyl xanthine, with the systematic name 1,3,7-trimethylxanthine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) [1-4]. It is widely present in beverages such as tea, coffee and cola, and occurs naturally in tea leaves, coffee beans, cocoa beans, kola nuts and guarana. In plants, caffeine acts as a natural pesticide by paralyzing and deterring insects that feed on the plant [5]. In its purified form, caffeine appears as a white crystalline powder with a strong bitter taste. Because of its central nervous system stimulant and mild diuretic properties, it is one of the most widely consumed psychoactive substances worldwide [6-8].

Caffeine is moderately soluble in water and highly soluble in organic solvents such as chloroform, dichloromethane and ethyl acetate. Its solubility in water increases in the presence of alkali and certain salts such as benzoates, citrates and salicylates [9-11]. These properties make liquid-liquid extraction a convenient technique for isolating caffeine from plant materials. In tea, caffeine is present along with tannins, pigments, proteins and other phytochemicals, and therefore requires selective extraction and purification before analysis [12-14].

The amount of caffeine in beverages varies depending on the source and processing method. Brewed coffee generally contains about 60-100 mg of caffeine per 100 mL, tea contains approximately 18-53 mg per 100 mL, while cola and cocoa contain comparatively lower amounts [15-17]. Moderate consumption of caffeine can improve alertness and reduce fatigue; however, excessive intake may lead to restlessness, insomnia, irritability and dependence [18-20]. Decaffeination processes are therefore employed in some beverages to reduce caffeine content [21].

Extraction of caffeine from tea is a common laboratory experiment that demonstrates principles of organic extraction, purification and characterization.

Creative Commons (CC BY-NC-SA 4.0)

This is an open access journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International](https://creativecommons.org/licenses/by-nc-sa/4.0/) Public License, which allows others to remix, tweak, and build upon the work noncommercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Address for correspondence:

Asmita M. Patil, Caffeine Department of Chemistry Sheth. J. N Paliwala College, Pali- Sudhagad-Raigad, M.S

Email: asmitapatil@jnpaliwala.in

How to cite this article:

Patil, A. M. (2026). Isolation and Quantitative Evaluation of Caffeine from Different Commercial Tea Powders Available in Raigad District, Maharashtra. *Bulletin of Nexus Journal*, 3(2), 76-81.
<https://doi.org/10.5281/zenodo.19848310>

Techniques such as liquid-liquid extraction, sublimation, melting point determination and thin layer chromatography (TLC) are widely used for the isolation and identification of caffeine [22–24]. The present study focuses on the extraction and comparative evaluation of caffeine from different commercial tea powder brands marketed in Raigad district, Maharashtra. In addition, selected physicochemical parameters such as pH, colour, taste, bulk density and normality of aqueous extract were analysed to assess the quality of tea samples. This study provides useful information regarding caffeine content and quality assessment of commercial tea powders and demonstrates a simple and reliable laboratory method suitable for academic and analytical purposes [25].

Materials and Methods

Different commercial varieties of tea powder were procured from the local market for analysis. The samples were stored in airtight containers at room temperature until further use. The study involved the evaluation of selected physical and physicochemical properties of tea powders before caffeine extraction. All chemicals used were of analytical grade, and distilled water was used throughout the experiment.

Physical Properties of Tea Samples

a. Taste and pH

One gram of each tea sample was taken in a clean beaker, and 100 mL of distilled water was added. The mixture was heated to boiling and then allowed to stand for about 5 minutes with the beaker covered. The infusion obtained was cooled slightly, and the taste of each tea sample was noted for comparative assessment. The pH of the filtrate was measured using a calibrated digital pH meter.

b. Analysis of Colour

One gram of each tea sample was taken in separate beakers, and 100 mL of distilled water was added. The mixtures were boiled for about 5 minutes, cooled and then filtered through filter paper. The colour of the filtrate was visually observed and recorded for each tea sample to compare the appearance and intensity of colour.

c. Determination of Bulk Density

A known mass of tea powder was accurately weighed and transferred into a clean, dry graduated measuring cylinder. The cylinder was tapped gently until a constant volume was obtained. This volume was noted as the bulk volume of the tea powder. The procedure was repeated for all tea samples. Bulk density was calculated using the formula:

$$\text{Bulk density} = \frac{\text{Mass of tea powder}}{\text{Bulk volume}}$$

The values obtained were compared to evaluate packing characteristics and the quality of tea powders.

d. Determination of Normality of Tea Extract

One gram of tea powder sample was taken in a beaker containing 100 mL of distilled water. The mixture was heated for about 10 minutes to prepare the extract and then filtered. The filtrate was transferred into a 250 mL standard volumetric flask and diluted up to the mark with distilled water. From this solution, 10 mL of tea extract was pipetted into a conical flask, a few drops of phenolphthalein indicator were added, and the solution was titrated against N/50 sodium hydroxide (NaOH) solution until a faint pink endpoint was obtained. The same procedure was followed for all tea samples, and the normality of the extract was calculated. These preliminary analyses were carried out to compare the quality and physicochemical characteristics of different commercial tea brands before proceeding to caffeine extraction and characterization.

Isolation

Isolation of caffeine from tea leaves is challenging because caffeine is not present alone; it occurs along with several other natural substances that must be separated during extraction. The major components of tea leaves include cellulose, caffeine, tannins, flavonoid pigments and chlorophyll. Cellulose is the structural material of plant cells and is insoluble in water, so it does not interfere significantly in the extraction process. Caffeine is one of the main water-soluble alkaloids present in tea leaves and may constitute up to about 5% of the dry leaf material. Tannins are high-molecular-weight polyphenolic compounds responsible for the colour and astringency of tea; they are acidic and must be removed during purification. Flavonoid pigments are water-soluble coloured compounds widely distributed in plants, while chlorophyll is the green photosynthetic pigment that may also be present. Effective isolation of caffeine, therefore, requires separation of these accompanying substances using suitable extraction and purification techniques.

Fifty grams of powdered tea sample was taken in a 600 mL beaker, and 250 mL of distilled water was added. The mixture was gently boiled for about 30 minutes to obtain an aqueous extract of tea. The hot extract was filtered through muslin cloth to remove insoluble plant material. To the clear filtrate, a solution of basic lead acetate was added carefully with stirring until no further

precipitate was formed. This step helps in the removal of tannins and other interfering substances present in the extract.

The mixture was then heated to boiling and filtered through a Buchner funnel under vacuum to obtain a clear solution. The filtrate was again heated to boiling and dilute sulphuric acid

was added slowly until a precipitate of lead sulphate formed. This step removes excess lead ions from the solution. The mixture was filtered once more to obtain a clear aqueous solution containing caffeine, which was then used for further extraction and purification steps in the isolation of caffeine from tea leaves.

Table 1: Separation of Caffeine from Tea

Tea Leaves	Process	Components
Tea leaves	Extraction with H ₂ O (100 °C)	Cellulose, Caffeine, Chlorophyll, Tannic acids, Flavonoids
H ₂ O insoluble fraction	Filtration	Cellulose, Chlorophyll, Tannic acids, Flavonoid pigments
H ₂ O soluble fraction	Contains caffeine	Caffeine is present in the aqueous layer
Extraction with chloroform	Liquid-liquid extraction	Caffeine transferred to the chloroform layer
Impurities removed	Separation step	Chlorophyll, Tannic acid, and flavonoid pigments were removed
Final product	Purification	Isolated caffeine

Table 1 represents the systematic separation of caffeine from tea leaves using aqueous extraction followed by organic solvent extraction

Characterization of Isolated Caffeine

The isolated caffeine was characterized using various physical and chemical methods to confirm its identity and purity.

a. Solubility Study

A small quantity of the isolated compound was taken in separate test tubes, and its solubility was tested in different solvents such as water, ethanol, methanol, ethyl acetate, chloroform and carbon tetrachloride. The solubility behaviour of caffeine in these solvents was observed and recorded using the gravimetric method.

b. Melting Point Determination

A small amount of the extracted caffeine was filled in a capillary tube, and the melting point was determined using a melting point apparatus. The melting point values obtained for different tea samples were compared with the standard melting point of caffeine.

c. pH of 1% (w/v) Solution

A 1% (w/v) solution of the isolated caffeine was prepared in distilled water. The pH of the solution was measured using a calibrated digital pH meter. The procedure was repeated for caffeine obtained from different tea samples.

d. Determination of Ash Content

About 1 g of the isolated caffeine sample was accurately weighed and transferred into a previously ignited and weighed porcelain crucible. The sample was heated in a muffle furnace at about 450 °C for 2–3 hours until a constant weight was obtained. After cooling in a desiccator, the crucible was weighed. The ash

percentage was calculated, and the procedure was repeated for all samples.

e. Thin Layer Chromatography (TLC)

The purity of the isolated caffeine was checked by thin-layer chromatography using silica gel plates. The sample and standard caffeine solution were spotted on the TLC plate and developed in a suitable solvent system. The plate was then placed in an iodine chamber for visualisation of spots. The R_f value was calculated and compared with that of standard caffeine.

f. Chemical Tests

i) Murexide Test: A small quantity of the isolated sample was taken in a watch glass and mixed with 2–3 drops of concentrated hydrochloric acid. A few crystals of potassium chlorate were added, and the mixture was evaporated to dryness in a water bath. After cooling, a drop of dilute ammonia solution was added. The development of a purple colour confirmed the presence of caffeine. The addition of dilute sodium hydroxide further intensified the colour.

ii) Iodine Test: To about 5 mL of a saturated solution of caffeine, a few drops of iodine solution were added, followed by a few drops of dilute hydrochloric acid. The formation of characteristic colour or precipitate indicated the presence of caffeine.

These characterization tests confirmed the identity and purity of caffeine isolated from different tea powder samples.

g. Derivative preparation

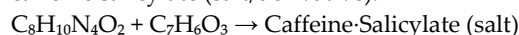
Preparation of Caffeine–Salicylic Acid Derivative

To confirm the identity of the isolated caffeine, a derivative in the form of caffeine salicylate was prepared. An equimolar quantity of salicylic acid was added to the purified caffeine present in a round-bottom flask. About 2–3 mL of chloroform was added, and the mixture was gently heated until the solids dissolved completely. The flask was removed from the heat, and petroleum ether was added dropwise until a white precipitate just began to form. A few drops of chloroform were added again to dissolve the precipitate and obtain a clear solution. The solution was allowed to cool slowly to room temperature and then kept in an ice bath to promote crystallization.

The crystals formed were collected by vacuum filtration using a Hirsch funnel and washed with a small amount of cold petroleum ether. The crystals were dried, weighed, and their melting point was determined. The derivative obtained (caffeine salicylate) shows a sharp melting point and confirms the identity of caffeine. The product was finally transferred to a labelled vial for record.

Reaction

Caffeine (base) reacts with salicylic acid to form caffeine salicylate (salt/derivative):



Caffeine acts as an organic base and accepts a proton from salicylic acid, forming an ionic derivative known as caffeine salicylate, which crystallizes readily and is useful for characterization.

Results and Discussion

Preliminary evaluation of different commercial tea powders was carried out by analysing parameters such as taste, pH, colour, bulk density and normality of the aqueous extract. The results indicated noticeable variation among the samples. The tea sample from Hasmukhrai & Co. showed a comparatively higher pH value, whereas the local brand exhibited a lower pH value. Most of the analysed tea samples possessed a bitter to strongly bitter taste and displayed a brown to dark brown colour, indicating the presence of typical tea constituents such as tannins and pigments. These observations provide useful comparative information regarding the quality and physicochemical characteristics of the different tea powder brands.

Table 2: Physical Characteristics of Different Tea Powder Samples

Sr. No.	Sample Name	Taste	pH	Colour	Bulk Density (g/mL)	Normality of Aqueous Extract
1	Hasmukhrai & Co.	Strong bitter	5.7	Dark brownish	0.48	0.0180
2	Red Label	Strong bitter	5.4	Dark brownish	0.49	0.0168
3	Taaza Tea	Bitter	4.3	Reddish brown	0.36	0.0124
4	Taj Mahal Tea	Bitter	4.6	Brownish	0.37	0.0150
5	Tata Tea Gold	Mild bitter	4.9	Dark brownish	0.42	0.0154
6	Tata Agni	Strong bitter	5.0	Reddish brown	0.42	0.0152
7	Wagh Bakri	Bitter	4.6	Brownish	0.43	0.0144
8	Local Brand	Bitter	4.1	Brownish	0.36	0.0130

The table.2 shows the comparative physical and physicochemical characteristics of different commercial tea powder samples analysed in the present study.

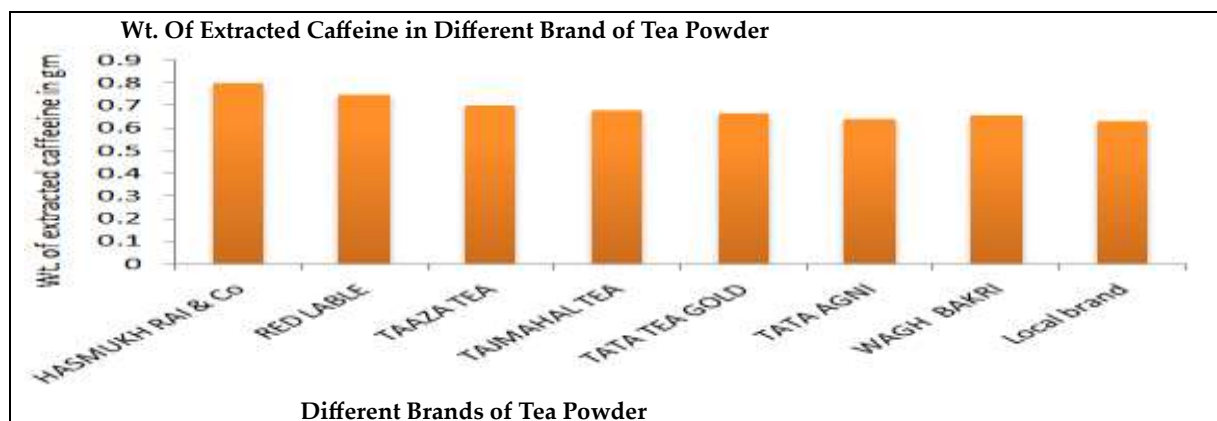
Table 3: Isolation of Caffeine from Different Tea Powder Samples and Other Parameters

Sr. No.	Sample Name	Weight of Extracted Caffeine (g)	Solubility Order	Melting Point (°C)	pH of 1% w/v Solution	% Ash	Rf Value
1	Hasmukhrai & Co.	0.80	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	6.0	0.041	0.80
2	Red Label	0.75	Chloroform > Carbon tetrachloride > Ethyl acetate >	237.7	5.8	0.039	0.78

Sr. No.	Sample Name	Weight of Extracted Caffeine (g)	Solubility Order	Melting Point (°C)	pH of 1% w/v Solution	% Ash	Rf Value
			Water > Ethanol > Methanol				
3	Taaza Tea	0.70	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.5	0.051	0.76
4	Taj Mahal Tea	0.681	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.5	0.034	0.75
5	Tata Tea Gold	0.665	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.4	0.036	0.75
6	Tata Agni	0.640	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.5	0.040	0.75
7	Wagh Bakri	0.660	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.5	0.034	0.76
8	Local Brand	0.634	Chloroform > Carbon tetrachloride > Ethyl acetate > Water > Ethanol > Methanol	237.7	5.7	0.033	0.76

The table.3 presents comparative data on caffeine yield and characterization parameters obtained from different commercial tea powder samples.

Graph 1 shows the variation in extracted caffeine content among different brands of tea powder.



Conclusion

Caffeine was successfully extracted and identified from different tea powder samples. Among all brands, HASMUKHRAI & CO. tea showed the highest amount of extracted caffeine. The melting point (≈ 237.7 °C) and pH (5.4–6.0) were found to be close to standard caffeine values. Solubility studies indicated that caffeine is most soluble in chloroform, confirming it as a suitable solvent for extraction and purification. The identity and purity of caffeine were confirmed by TLC, Murexide test (purple-red colour), and iodine test. The derivative showed a melting point of about 137.7 °C. Overall, the study confirms

successful extraction, purification and characterization of caffeine from tea samples and shows variation in caffeine content among different brands.

Acknowledgement

The author sincerely expresses gratitude to the Principal of the institution, **Dr. S. C. Lahupachang**, for continuous encouragement and for providing a supportive academic environment. The author also gratefully acknowledges **Prof. Satu Chavhan** for valuable guidance, support and motivation throughout the research work. The assistance and resources provided by the institutional library are also thankfully

acknowledged, as they were invaluable for the successful completion of this study

References

1. Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). *Food Chemistry* (4th ed.). Springer, Berlin.
2. Clarke, R. J., & Macrae, R. (1985). *Coffee: Chemistry, Vol. 1*. Elsevier Applied Science, London.
3. Ashihara, H., Sano, H., & Crozier, A. (2008). Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. *Phytochemistry*, 69(4), 841–856.
4. Spiller, G. A. (1998). *Caffeine*. CRC Press, Boca Raton.
5. Nathanson, J. A. (1984). Caffeine and related methylxanthines: Possible naturally occurring pesticides. *Science*, 226(4671), 184–187.
6. Fredholm, B. B., et al. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews*, 51(1), 83–133.
7. Rang, H. P., Dale, M. M., Ritter, J. M., & Flower, R. (2012). *Rang and Dale's Pharmacology* (7th ed.). Elsevier.
8. Goodman, L. S., & Gilman, A. (2011). *The Pharmacological Basis of Therapeutics* (12th ed.). McGraw-Hill.
9. Dean, J. A. (1999). *Lange's Handbook of Chemistry* (15th ed.). McGraw-Hill.
10. Furniss, B. S., Hannaford, A. J., Smith, P. W., & Tatchell, A. R. (1989). *Vogel's Textbook of Practical Organic Chemistry* (5th ed.). Longman.
11. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Engel, R. (2015). *Introduction to Organic Laboratory Techniques* (5th ed.). Cengage Learning.
12. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Springer.
13. Skoog, D. A., Holler, F. J., & Crouch, S. (2014). *Fundamentals of Analytical Chemistry* (9th ed.). Cengage.
14. Robinson, T. (1991). *The Organic Constituents of Higher Plants* (6th ed.). Cordus Press.
15. Mendham, J., Denney, R. C., Barnes, J. D., & Thomas, M. (2000). *Vogel's Quantitative Chemical Analysis* (6th ed.). Prentice Hall.
16. Sethi, P. D. (1996). *Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations*. CBS Publishers.
17. Camargo, M. C. R., & Toledo, M. C. F. (1999). Caffeine content of commercial beverages. *Food Control*, 10, 337–339.
18. Bunker, M. L., & McWilliams, M. (1979). Caffeine content of common beverages. *Journal of the American Dietetic Association*, 74, 28–32.
19. Nawrot, P., et al. (2003). Effects of caffeine on human health. *Food Additives and Contaminants*, 20(1), 1–30.
20. Smith, A. (2002). Effects of caffeine on human behavior. *Food and Chemical Toxicology*, 40(9), 1243–1255.
21. McGee, H. (2004). *On Food and Cooking: The Science and Lore of the Kitchen*. Scribner.
22. International Organization for Standardization (ISO). (2005). ISO 20481: Determination of caffeine content in coffee and tea.
23. Indian Standards Institution. (2003). IS 3633: Specification for Tea. Bureau of Indian Standards.
24. Somporn, C., Kamtuo, A., Theerakulpisut, P., & Siriamornpun, S. (2011). Quality and antioxidant properties of tea. *Food Chemistry*, 125, 133–139.
25. Crozier, A., Jaganath, I. B., & Clifford, M. N. (2009). Dietary phenolics: Chemistry and bioavailability. *Natural Product Reports*, 26, 1001–1043.