



J. Chem. Pharm. Res., 2010, 2(4):194-198

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Characterization of Caffeine Isolated from *Camellia Sinensis* Leaves of Sikkim Himalayan Region

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ABSTRACT

*The present study was conducted to isolate the caffeine from the *Camellia sinensis* (green tea) leaves obtained from the Sikkim Himalayan Region followed by its spectroscopic and HPLC studies. Caffeine (3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) was isolated from leaves using a liquid-liquid extraction method. Isolated product was evaluated by using TLC, UV-Visual Spectrophotometer, FTIR, NMR studies and HPLC. The data obtained from these studies was compared with standard caffeine. From above studies, it was concluded that the compound isolated from *Camellia sinensis* leaves is caffeine and having the characteristics almost similar to that of standard caffeine.*

Keywords: Caffeine, TLC, HPLC, NMR, FTIR.

INTRODUCTION

Herbs play a vital role in maintaining human health and contribute towards improvement of human life. Plants are considered as chemical laboratories capable of biosynthesizing number of bio-molecules of different chemical class. Many of them are proved to be precursors for development of other drugs. Furthermore many western drugs have their origin in plant extract. There are a number of herbal agents which are successfully used for GIT, CV, nervous and metabolic disorder [1]. Since last two decades, as herbal wave continues to dominate drug discovery and development. Recent advances in extraction, chromatography, hyphenated technique, screening of natural product research has necessitate sound knowledge of plant research. Rapid progress of biotechnology has opened new avenues to hasten natural products research [2].

Tea (*Camellia sinensis*, family Theaceae) is the most widely consumed plant-based beverage in the world. Composition of tea varies with species, season, age of leaves, climate, and horticultural practices. Black tea represents about 76% to 78% of the tea produced and consumed worldwide. Green tea represents about 20% to 22%, while oolong tea accounts for only about 2% of the world production of tea. Green tea is prepared from fresh tea leaves that are pan-fried or steamed and dried to inactivate enzymes [3]. Tea leaf contains a very large amount of polyphenols, which is the most specific feature of tea. These phenolics are catechins, flavanols, leucoanthocyanines and phenolic acids and their derivatives. Among them catechins are predominant. Caffeine and amino acids are also known as important components. Composition of caffeine varies from 2.5-3.0% in green tea [4].

Caffeine is most important naturally occurring xanthine derivative. About 100 mg of caffeine is contained in one cup of coffee or tea [5]. Caffeine is also used as a decongestant, increase in energy, weight loss, analgesic, appetite suppressant and diuretic [6,7,8].

EXPERIMENTAL SECTION

Materials

Caffeine was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All the other reagents and chemicals were purchased from Sigma chemicals, USA and S.D. Fine Chemical Ltd., Mumbai, India.

Methods

1. Gathering of Green Tea Leaves

Green tea leaves were collected from East Himalayan region and authenticated in Department of Pharmacognosy, Himalayan Pharmacy Institute, Majhitar, Sikkim. Material was washed, shade dried, powdered, passed through sieve no. 60 and stored in well closed air-tight containers for experimental work.

2. Isolation of Caffeine from Green Tea Leaves

Tea leaves were placed in a beaker and 1 litre water was added to it. Mixture was digested on a water bath for 15-20 minutes. It was then filtered through a muslin cloth. To the hot filtrate, a basic lead acetate solution was added when a precipitate was obtained. Addition of lead acetate was continued until no more precipitate was formed. Mixture was filtered while hot through a filter paper. Residue was washed with water. Filtrate was boiled and dilute sulphuric acid was added to precipitate lead sulphate, until no more precipitate appeared. Precipitates of lead sulphate were filtered off. Decolorizing carbon was added and filtrate was concentrated by evaporation to about 300-400 ml. Filtrate was filtered and shaken with about 75 ml of chloroform in a separating funnel and chloroform layer was separated. Extraction was repeated twice. Chloroform layer was collected and solvent was distilled off. Residue obtained was then re-crystallized with ethanol [9].

3. UV-Visual Spectrophotometer

Ultraviolet Spectra were recorded using Shimadzu UV-Vis Spectrometer, Model 1700S, and chloroform was used as solvent for the dilution of sample as well as blank. The λ_{\max} value of test sample and standard was recorded [10,11].

4. Thin Layer Chromatography (TLC)

Purity of isolated compound (caffeine) was checked using TLC method and compared with standard. The test sample and standard was dissolved in chloroform and applied to pre-coated TLC plates with Silica gel (Merck, 60F) using Chloroform: acetone: methanol (30:30:30 v/v/v)

solvent system and detection was done by putting TLC plates in iodine chamber. Then R_f value was calculated [12-15].

5. FTIR Spectra

Infrared spectra of isolated compound were recorded in the range of 4000 – 400 cm^{-1} using potassium bromide on Shimadzu FTIR model-8400 spectrophotometer and compared with standard FTIR spectra [15]. The KBr pellet technique was used for this study. Each KBr pellet were contains not more than 2% isolated compound [14,16].

6. Nuclear Magnetic Resonance (NMR) Spectra

^1H NMR and ^{13}C NMR spectra were recorded using ADVANCE DPX 200 FTNMR spectrometer from Central Drug Research Institute, Lucknow. The chemical shifts are reported in δ (ppm) downfield from tri-methyl silane (TMS), which was used as internal standard. Cadmium chloride (CDCl_3) was used as solvent for this study [11,17].

7. High Performance Liquid Chromatography (HPLC) Analysis

Peak area of isolated compound was compared with the peak area of standard caffeine (obtained from SD Fine Chemicals, Mumbai, India). Analysis was performed using 50 $\mu\text{g/ml}$ concentration of isolated and standard compound in methanol: glacial acetic acid (95:5 v/v) solvent.

Analysis of the compounds was performed by recording the peak area on Shimadzu HPLC model LC-20 AT using water: methanol: Acetic acid (70:27:3 v/v/v) at flow rate 1.0ml/min. Twenty micro liters (20 μl) of injection volume was eluted in RP C (18) column (4.6 \times 150 MM) at room temperature and was monitored at wavelength (λ_{max}) 273 nm using diode array UV detector. The retention time and peak area of isolated compound was compared with standard. Before the performance of HPLC analysis, solid phase extraction was performed to avoid the blockage of column from unwanted material [14,17,18,19].

RESULTS AND DISCUSSION

1. Comparisons of TLC, HPLC and UV Spectroscopic study of isolated compound with Standard of Isolated Compound

UV-Visual Spectroscopic study and Thin Layer Chromatography (TLC) of the isolated compound were found almost similar to that of the standard caffeine, so this study concludes that the isolated compound may be caffeine [12,13,14,15].

Table 1. Comparisons of TLC, HPLC and UV Spectrophotometer study of isolated compound with Standard

Sample	UV-Spectra (λ_{max} in nm)	TLC Studies (R_f)	HPLC Studies		
			Peak Area (Mv.s)	Retention time (min)	Percentage Purity (%)
Test	273	0.66	1410.62	8.12	99.27
Standard	273	0.66	1406.27	8.12	-----

From HPLC studies was confirmed that the isolated compound is caffeine, because the peak area and retention time of this compound was matched with the peak area and retention time of

standard caffeine. By using HPLC method, the percentage purity of isolated caffeine was also calculated [14,17,18,19]. All data for above given studies are given in Table 1.

2. FTIR Spectra

From FTIR studies found that the corresponding spectra all the functional groups are within the range and IR spectra of isolated caffeine almost matched with the spectra of standard caffeine. This study concludes that the isolated compound was caffeine [14,16]. Corresponding spectra for isolated caffeine and the standard caffeine are given in Table 2.

Table 2. FTIR Spectra of the Isolated Compound and Standard Caffeine

Bonds	Isolated Compound (cm ⁻¹)	Standard Caffeine (cm ⁻¹)
C=O Str.	1703.20	1707.06
C-C Str.	974.08	974.08
C-H bend.	1361.79	1359.86
C-H Str.	2953.12	2955.04
C-N Str.	1546.96	1548.89
N-H Str.	3111.28	3111.28

3. ¹H and ¹³C-NMR Spectra

The structure of the Caffeine under investigation was further supported by the ¹H and ¹³C-NMR spectral measurement. The ¹H and ¹³C-NMR spectra data of the isolated compound is recorded and assigned in the Table 3 and Table 4, respectively. From this study, it was concluded that the isolated compound is caffeine, because the NMR spectra conforms the caffeine structure [11,16].

Table 3. Observation Table for NMR Spectra (Proton Spectra) of isolated Compound

Groups	Chemical Shift (δ) ppm
1-N-CH ₃	3.59 s
3-N-CH ₃	3.41 s
7-N-CH ₃	4.00 s
8-H	7.51 s

Table 4. Observation Table for NMR Spectra (¹³C-NMR Spectra) of Isolated Compound

Groups	Chemical Shift (δ) ppm	Multiplicity
C-2	151.52	s
C-4	148.53	s
C-5	107.39	s
C-6	155.21	s
C-8	141.28	d
C-10-CH ₃	29.54	q
C-11-CH ₃	27.72	q
C-12-CH ₃	33.39	q

Acknowledgements

Authors are thankful to Himalayan Pharmacy Institute, Majhitar, Sikkim, India for providing facilities to carry out that research work. Authors are also thankful to CDRI, Lucknow, India for NMR studies.

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