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Isolation, Identification, and Quantitative Evaluation of Caffeine from the Young Leaves of Green Tea (*Camellia sinensis* L.)

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Abstract

Background: Caffeine is a naturally occurring purine alkaloid that belongs to methylxanthine derivative. Caffeine exerts varying chemical and biological effects on humans. commonly characterized by central nervous system stimulant, qualities, and the ability to lower the risk of cardio metabolism and pain-relieving properties. It is mostly derived from different plants including Coffee arabica and *Camellia sinensis* (family Theaceae) which are also abundant in catechins and polyphenols.

Objective: This study aimed to investigate, isolate, and estimate the caffeine from green tea through a series of extraction and analytical techniques.

Methods: Green tea extracts underwent phytochemical screenings like flavonoids, alkaloids, terpenes, saponins, Tannins, and specific murexide chemical tests. Moreover, chromatographic and spectroscopic investigations were used to identify the isolated compound.

Results: The findings show the isolation of 0.097% of caffeine was successfully separated and isolated from the leaves of *Camellia sinensis*.

Conclusion: These results demonstrate that Camellia sinensis contains a significant amount of caffeine, supporting its role as a major dietary source of bioactive purine alkaloids.

عزل وتحديد وتقييم الكافيين من الأوراق الشابة للشاي الأخضر (*Camellia sinensis* L.)

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الخلاصة

الكافيين هو قلويد بيوريني طبيعي ينتمي إلى مشتقات الميثيل زانثين، وله تأثيرات كيميائية وبيولوجية متفاوتة على البشر. يُعرف الكافيين غالبًا بكونه منبّهًا للجهاز العصبي المركزي، بالإضافة إلى خصائصه في تقليل خطر الاضطرابات القلبية الأيضية وتسكين الألم. يُستخلص الكافيين بشكل رئيسي من عدة نباتات، منها Coffea arabica وCamellia sinensis (من عائلة Theaceae)، والتي تحتوي أيضًا على كميات وفيرة من الكاتيشينات والبوليفينولات. هدفت هذه الدراسة إلى التحري، وعزل، وتقدير كمية الكافيين في الشاي الأخضر من خلال سلسلة من تقنيات الاستخلاص والتحليل. خضعت مستخلصات الشاي الأخضر لاختبارات كيميائية نباتية أولية (Phytochemical Screening) شملت فحص وجود الفلافونويدات، والقلويدات، والتربينات، والصابونينات، والعفص (Tannins)، بالإضافة إلى اختبارات كيميائية نوعية باستخدام كاشف الموريكساید لتحديد الكافيين. كما استُخدمت تقنيات التحليل الكروماتوغرافي والتحليل الطيفي للتعرف على المركب المعزول. أظهرت النتائج أنه تم عزل وفصل الكافيين بنسبة 0.097% من أوراق Camellia sinensis بنجاح. تُظهر هذه النتائج أن Camellia sinensis يحتوي على كمية ملحوظة من الكافيين، مما يدعم دوره كمصدر غذائي رئيسي للقلويدات البيورينية النشطة بيولوجياً.

Introduction

The leaves and buds of *Camellia sinensis* have a long history originating from China and other countries and are used to make the popular beverage known as tea [(1)]. Theaceae is the family that includes the flowering plant genus Camellia. However, all teas are made by subjecting them to varying degrees of oxidation during processing. Countries in central Africa, Sri Lanka, Indonesia, Taiwan, India, China, and Southeast Asia are the primary tea producers [(2)]. The tea plant is a perennial shrub with White, fragrant flowers bloom alone or in groups of two to four. The undersides of the glossy, verdant leaves of the tea plant are often hairy as in Figure 1. The brownish-green fruits have characteristic spherical or flattened seeds [(3)]. In vitro, the phytochemicals and tea extracts and were reported to inhibit neuronal damage after cerebral ischemia, Inhibiting bacterial skin activity. Also, scavenging free radicals, and increasing the activity of antioxidant enzymes [(4-6)]. Furthermore, in vivo, studies reported the major phytochemicals cause Improvement in cognitive impairment, act as Protection against hepatotoxicity, and revealed they can Improve glucose metabolism [(7)].The importance of tea belongs to the presence of a variety of chemical composition classes. addition to primary metabolites, it is characterized by pseudo-alkaloids such as caffeine, theobromine, and theophylline. Moreover, the presence of gallic acids, Ellagic acid, Caffeic acid, polyphenols, myricetin glycosides, Anthocyanins, quercetin, tannic acid, Catechins, and their derivatives for health benefits [(8)] Caffeine, Figure 2, a purine alkaloid, is a natural trimethyl xanthine alkaloid regulated by various plant enzymes and rapidly absorbed and distributed throughout the body. The main mechanism is the blocking of adenosine receptors. The Tolerance of caffeine can progress with continuous use [(9)]. This research intends to determine, separate, identify, and estimate the quantity of caffeine found in green tea leaves.

Figure 1: leaves of green tea (3)

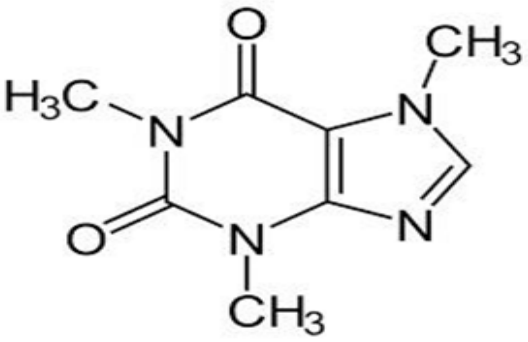

Figure 2: chemical structure of caffeine (7)

Material and methods

Plant material

The dried young Leaves of the Green tea plant was collected from markets in Baghdad, Iraq in October (2023). The leaves were then ground into a fine powder and weighed.

Reference compound and reagents

Caffeine was purchased from Chengdu Biopurify Phytochemicals Ltd, china. All The solvents with purity > 99%. Chloroform was purchased from Merck, USA. additional chemical solvents from Thermo Fisher Scientific Chemicals Co. The solid chemical was from Weifang Boteng Chemical Co., Ltd.

Qualitative screening for secondary metabolites of green tea leave

The decocted extracts of *Camellia sinensis* leaves were tested regarding optimum measures of qualitative examination to detect the foremost classes of plant secondary metabolites [10-12].

Detection of Saponins (Foam Test)

A test tube was filled with 5 mL of distilled water and 1 mL of aqueous leaf extract. The mixture was then agitated vigorously for several minutes. The foam test produced a stable foam layer exceeding 1 cm in height, confirming the presence of saponins in the aqueous leaf extract.

Detection of Terpenoids (Salkowski Test)

In general, for phytochemical screening, leaf extract was prepared from the decoction of 20 gm of dried leaves with 100 mL methanol for one week. Approximately 5 mL of the extract was mixed with three milliliters of chloroform. Along the side of the test tube, we added about two milliliters of concentrated sulfuric acid. a distinct reddish-brown coloration developed at the interface between the chloroform and acid layers, indicating a positive result for terpenoids.

Detection of Flavonoids

Approximately 2–3 mL of alcoholic potassium hydroxide (KOH) solution was added to 1 mL of the methanolic plant extract. The change in color development and appearance of a yellow coloration were taken as evidence of the presence of flavonoid compounds.

Detection of Alkaloid

A 3.0 mL aliquot of the methanolic extract was treated and acidified with a few drops of nonoxygenated acid (hydrochloric acid). Subsequently, the Wagner reagent of the experiment was prepared, dissolving iodine in potassium iodide, and a few drops were added. The formation of a reddish-brown precipitate was considered indicative of alkaloid presence.

Detection of Tannins

Tannins are a class of polyphenolic compounds. however, this test depends on the reaction of polyphenolics with ferric ions of $FeCl_3$ solution. in this procedure, about 3 ml of leaf extract (0.23 g extracted in 10 mL of distilled water and filtered) was treated with one percentage ferric ion solution. Observation of a change in color intensity (dark green or dark blue) confirms the presence of tannins.

Isolation of caffeine from the leaves of green tea

The powdered leaves of *Camellia sinensis L* were about **(**59.7 g), as seen in Figure 3. The

plant materials were extracted with 280 ml of distilled water using a heater with continuous stirring for 20 min, then using filter paper to filtrate the hot extract to pass the dissolved caffeine crystals. A basic solution of lead-acetate was added to the filtrate to prevent the formation of any precipitate. Sodium carbonate was added to the aqueous extract, after which the mixture was partitioned and the caffeine was separated with chloroform using a separatory funnel. The caffeine, more soluble in the organic phase, migrated into the chloroform layer [13,14]. Following separation, the chloroform was evaporated, leaving behind purified caffeine crystals. The isolated caffeine crystals were weighed quantified and subjected to the Murexide test and FTIR.

The murexide test for isolated caffeine

Sufficient amounts of potassium chlorate powder were added to a few caffeine crystals in a small beaker, and treated with three drops of concentrated HCL. The mixture was evaporated to dryness on the heater. After that, it was subjected to ammonia vapor to give a positive result [15].

Thin Layer Chromatography TLC

Analysis of isolated crystals using TLC (G_f 245) compared with the caffeine standard was performed and UV detection at 245nm of the expected caffeine. The development was done in Three mobile phases as follows:

S_a: Acetone:Water: Ammonia (9:0.7:0.3) [16]

S_b: Methanol: NH4OH (100:1) [17]

S_c: Isopropanol:Acetic Acid: H_2O (6:2:2) [18]

Spectral analysis (FTIR) for Isolated compound

An isolated compound was subjected to FTIR (Scanning range was 4000-400 cm, Shimadzu Co. 8300, Japan) for structural elucidation of the main functional groups [19]. Work done at the College of Pharmacy / Mustansiryiah University. The compound was analyzed using a sufficient quantity of KBr and compressed into a disk.

Results and discussions

Phytochemical screening

The results confirmed the presence of phytochemicals such as saponin, flavonoids, alkaloids, tannins, and terpenes which are beneficial in the biological actions of the *Camellia sinensis* plant. As seen in the Table 1.

Isolation of caffeine from the leaves of green tea

The resulting crystals were crushed, recrystallized with chloroform, and then weighted by sensitive balance. Caffeine is a xanthine alkaloid that can detected with reliable murexide tests. The appositive result shows pink crystals, as shown in Figure 4 and Figure 5.

Estimation of caffeine

About 56.8 mg of caffeine as white crystals isolated from 59 gm leaves of *Camellia sinensis* and approximately estimated 96.55mg/100g ≈ 0.097% (w/w) as below:

$$\textbf{Concentration (mg/100g)} = \left(\frac{56}{58}\right) \times 100 = 96.55\text{mg}/100 \approx 0.097\%$$

Generally, tea and coffee typically have high concentrations of caffeine and polyphenols. However, the caffeine content in various tea extracts varied from geographical regions; Turkey, China, and India 51.80, 51.6, and 10.32 mg/g, respectively. Pharmacologically, stimulating the release of adrenaline from Caffeine affects the central nervous system and improves alertness and cognitive function [20].

Thin Layer Chromatography (TLC)

The isolated crystals gave the same R_f value (Retention factor) of caffeine standard (0.78). using S_a as the mobile phase in the study as shown in Figure 6**.**

Fourier Transform Infrared Spectrometry (FTIR)

Structure elucidation by FT-IR for isolated caffeine showed the presence of several functional groups in specific regions as shown in Figure 7 and Table 2. The spectra data are compatible with previously informed

literature for the isolated caffeine crystals [21, 22].

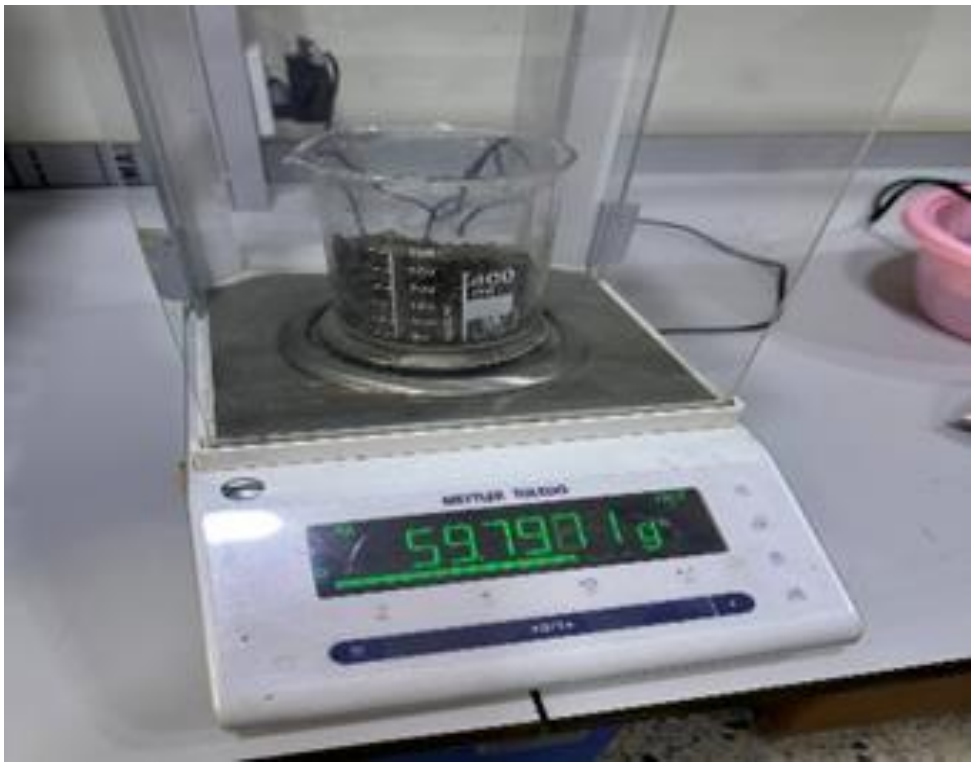

Figure 3: Crude of *Camellia sinensis*

Table 1: phytochemical detection of *Camellia sinensis* leaves

phytochemical tests	Observation	Inference
Saponins	stable foam layer. 1 cm	present
Terpenes	Reddish-brown interface	present
Tannins	Dark green in color	present
Flavonoid	Yellow color	present
Alkaloids	Redish-brown color	present

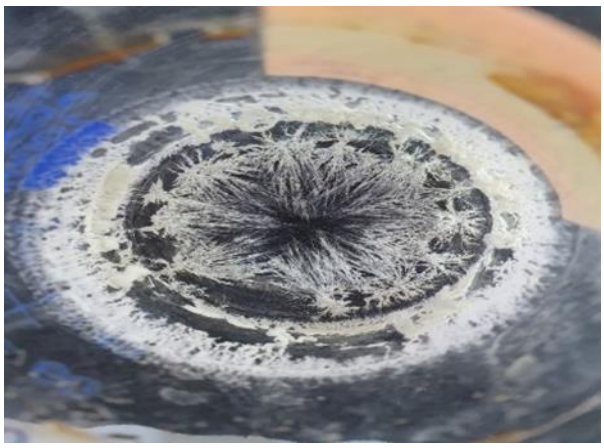

Figure 4: Caffeine crystals

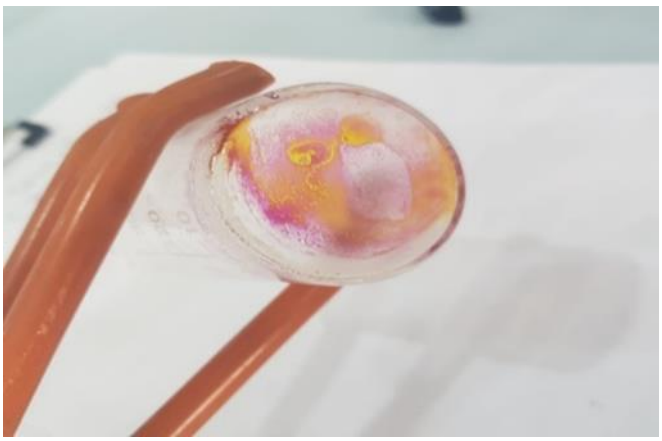

Figure 5: Isolated caffeine detection by murexide test

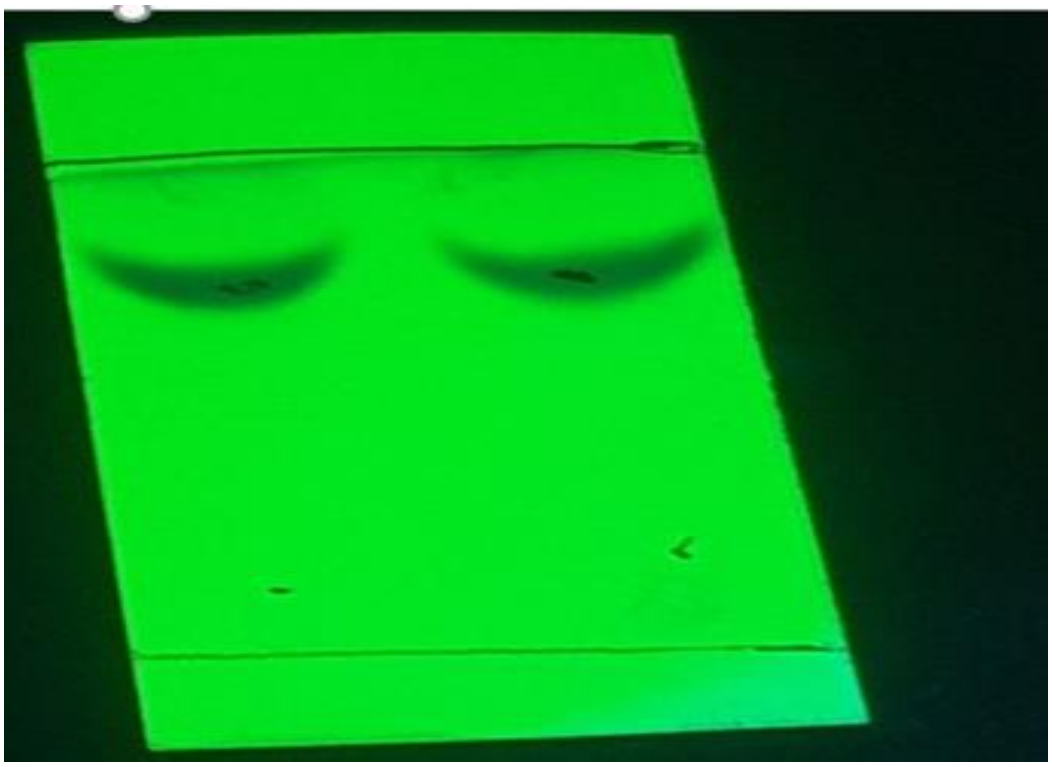

Figure 6: TLC for Detection of the isolated compound compared with the caffeine standard at Rf value of 0.78 under UV light 254 nm using mobile phase: Acetone: Water: Ammonia (9:0.7:0.3).

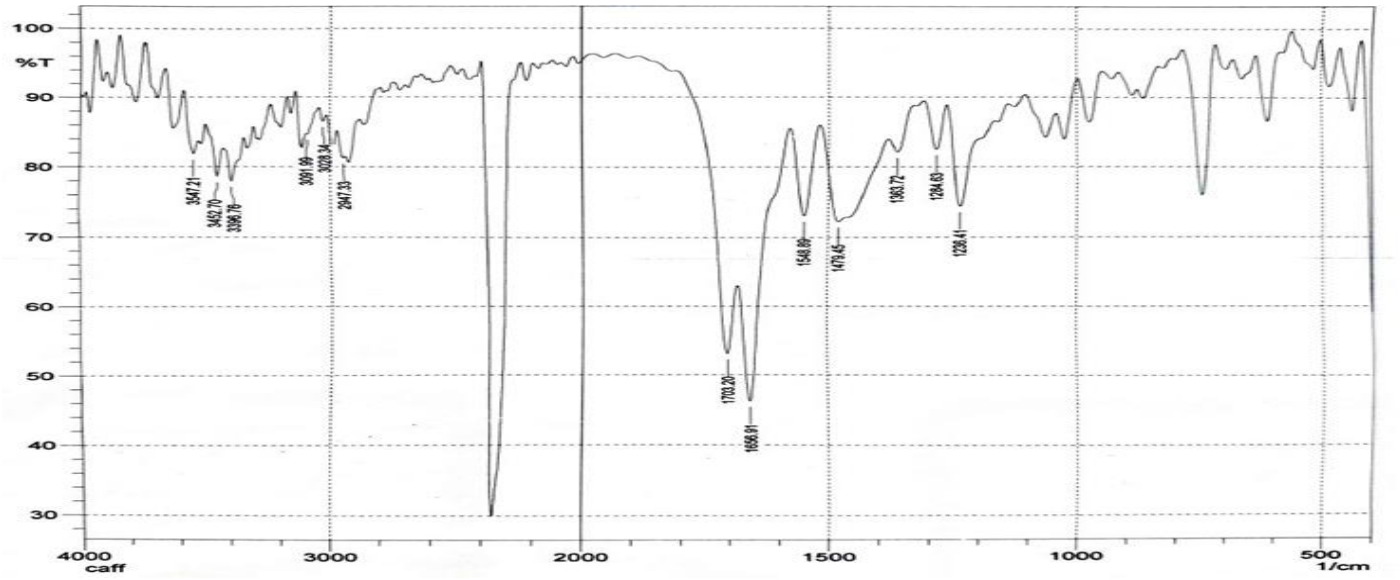

Figure 7: FT- IR spectrum analysis of the isolated compound

Table 2: FT-IR interpretation of isolated caffeine

Functional group	*Band (cm^{-1})*	*interpretation*
C-H	~3109	Aromatic C-H stretching
C-H	~2947-2850	Aliphatic C-H stretching
C=O	~1695	Stretching of the carbonyl group of amide
C-N	~1024,1188, 1238	Stretching band
C=N	~1598	Stretching of the imidazole ring
C-H	~758-600	Out-of-plane bending of aromatic

Conclusions

The current study used a solvent extraction method to successfully separate caffeine from young leaves of Camellia sinensis (green tea). It is considered a natural source of caffeine, producing a good concentration of 0.097% of pure caffeine from 59 g of dried plant material. The screening of plant material is used to detect important bioactive components like alkaloids, flavonoids, tannins, and saponins in addition to caffeine, beyond the potential health benefits and

underscoring the importance of tea drinking. Fourier Transform Infrared (FTIR) spectroscopy was used to establish the isolated caffeine identity; unique peaks were visible in the measured spectral data. However, the researchers in this work are recommended to identify and elucidate the separated caffeine by other spectroscopic analysis. Future Research may focus on the extraction of further bioactive compounds in the same plan and comparative analysis between common commercial sources of caffeine.

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Authors' contributions
T.A.A carried out the caffeine extraction and isolation and writing, the chromatographic and spectral analysis carried out by A.A.T, and participated in manuscript writing with T.A.A. Plant collection material and preliminary analysis and preparation done by M.H.A. All authors participated in designing and coordinating the study. All authors read and approved the final manuscript.

Conflict of interest: Authors do not have any conflict of interest to declare.

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