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Comparative study of different extraction methods for caffeine extraction and determination of the maximum yield

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Abstract:

Caffeine is a naturally occurring methylxanthine alkaloid extensively utilized in pharmaceutical formulations, functional foods, and beverages because of its stimulant and therapeutic properties. The effectiveness of caffeine isolation largely depends on the extraction technique employed. The present investigation was undertaken to compare five commonly used extraction methods—maceration, percolation, decoction, ultrasound-assisted extraction (UAE), and Soxhlet extraction—for the recovery of caffeine from *Camellia sinensis* leaves under comparable laboratory conditions. Equal quantities of powdered tea leaves were processed using water as the primary extraction medium, followed by purification through liquid-liquid extraction. The recovered caffeine was dried, weighed, and extraction yield was calculated to evaluate the efficiency of each method. Considerable variation in extraction performance was observed among the techniques. Maceration produced the lowest recovery because of slow solvent diffusion, whereas percolation and decoction resulted in moderate yields. Ultrasound-assisted extraction significantly improved caffeine recovery by enhancing cell disruption and mass transfer while reducing extraction time. Soxhlet extraction demonstrated the highest extraction efficiency, yielding **3.28%** caffeine, followed by UAE (**0.54%**), decoction (**0.40%**), percolation (**0.29%**), and maceration (**0.23%**). The overall extraction efficiency followed the order: **Soxhlet > Ultrasound-Assisted Extraction > Decoction > Percolation > Maceration**. These findings indicate that Soxhlet extraction remains the most effective technique for exhaustive laboratory-scale caffeine recovery, whereas UAE represents a promising rapid and energy-efficient alternative. The study provides practical information for selecting suitable extraction strategies in pharmaceutical, nutraceutical, and food-processing applications.

Keywords: Caffeine extraction, *Camellia sinensis*, Soxhlet extraction, Ultrasound-assisted extraction, Maceration, Percolation, Decoction, Extraction yield, Alkaloids, Pharmaceutical analysis.

Chapter 1: Introduction

1.1 Introduction of Caffeine

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring methylxanthine alkaloid that is widely distributed in several economically important plant species, including *Camellia sinensis* (tea), *Coffea arabica* (coffee), *Theobroma cacao* (cocoa), *Paullinia cupana* (guarana), and *Cola* species. In plants, caffeine functions as a natural defense compound by protecting against herbivores and certain microbial pathogens, while also influencing seed germination and plant growth. In humans, it is recognized as the most extensively consumed psychoactive substance worldwide because of its ability to stimulate the central nervous system, enhance alertness, reduce fatigue, and improve cognitive performance. Owing to its widespread occurrence in commonly consumed beverages, caffeine has become an integral component of daily dietary intake across diverse populations.

From a chemical perspective, caffeine belongs to the xanthine class of purine alkaloids, together with theophylline and theobromine. These structurally related compounds exhibit similar pharmacological properties, including central nervous system stimulation, bronchodilation, cardiac stimulation, and smooth muscle relaxation. The primary mechanism of action of caffeine involves competitive antagonism of adenosine receptors, which increases neuronal activity and promotes the release of neurotransmitters such as dopamine and norepinephrine. At higher concentrations, caffeine can also inhibit phosphodiesterase enzymes and modulate intracellular calcium release, thereby contributing to its diverse physiological and pharmacological effects.

The broad spectrum of biological activities associated with caffeine has generated considerable interest in pharmaceutical, biomedical, and food science research. Clinically, caffeine is incorporated into numerous pharmaceutical formulations, including analgesic combinations for the management of headache and migraine, respiratory stimulants for the treatment of apnea of prematurity, and selected weight-management preparations due to its thermogenic and metabolic effects. Furthermore, its capacity to enhance the efficacy of certain analgesics has led to its inclusion in several over-the-counter medications. Beyond therapeutic applications, caffeine is extensively utilized in the food and beverage industry as a naturally occurring stimulant in coffee, tea, energy drinks, carbonated beverages, and functional foods.

The increasing global demand for caffeine has highlighted the importance of developing efficient, economical, and environmentally sustainable extraction techniques. The recovery of high-purity caffeine from natural plant sources is influenced by several factors, including the type of raw material, solvent system, extraction temperature, duration of extraction, and the extraction method employed. Conventional methods such as maceration, percolation, decoction, and Soxhlet extraction remain widely used because of their simplicity and reliability, whereas modern techniques such as ultrasound-assisted extraction have gained attention for improving extraction efficiency while reducing solvent consumption, processing time, and energy requirements.

Because of its significant pharmacological value, commercial importance, and extensive industrial applications, caffeine continues to be one of the most thoroughly investigated natural alkaloids. Research on caffeine encompasses pharmaceutical chemistry, pharmacology, natural product chemistry, analytical science, toxicology, nutraceuticals, and process optimization. Comparative evaluation of different extraction techniques is therefore essential for identifying the most suitable method to maximize caffeine recovery while maintaining product quality and operational efficiency for laboratory-scale as well as industrial-scale applications.

1.2 History of Caffeine

The history of caffeine is closely intertwined with the evolution of two of the world's most popular beverages—tea and coffee. Long before caffeine was chemically identified, plants containing this naturally occurring alkaloid were widely consumed for their stimulating and medicinal properties. Historical records indicate that tea consumption originated in ancient China around 2737 BC, where it was traditionally valued for promoting alertness and well-being. Similarly, the use of coffee is believed to have begun in Ethiopia during the ninth century, from where it gradually spread to the Middle East and subsequently to Europe, becoming an important part of social and cultural traditions.

Although the physiological effects of caffeine-containing beverages had been recognized for centuries, the active compound responsible for these effects remained unknown until the nineteenth century. A major breakthrough occurred in 1819, when the German chemist Friedlieb Ferdinand Runge successfully isolated caffeine from coffee beans at the request of Johann Wolfgang von Goethe. This achievement marked the beginning of scientific investigations into the chemistry and pharmacology of caffeine.

Subsequent discoveries further advanced the understanding of this important alkaloid. In 1827, caffeine was independently isolated from tea leaves and was initially named "theine." Later studies confirmed that caffeine and theine were chemically identical compounds. A significant milestone was achieved in 1895, when the Nobel Prize-winning chemist Emil Fischer elucidated the complete chemical structure of caffeine and successfully synthesized it in the laboratory. His pioneering work laid the foundation for modern alkaloid chemistry and greatly expanded research into naturally occurring bioactive compounds.

Over the past century, caffeine has become one of the most extensively investigated natural alkaloids because of its pharmacological significance, therapeutic potential, and widespread industrial applications. Today, it is extensively utilized in pharmaceutical formulations, food and beverage products, nutraceuticals, cosmetics, and analytical research, making it a compound of considerable scientific and commercial importance.

1.3 Chemical Structure and Physicochemical Properties of Caffeine

Caffeine is a naturally occurring methylxanthine alkaloid belonging to the purine class of heterocyclic compounds. Chemically known as 1,3,7-trimethylxanthine, it consists of a xanthine nucleus substituted with three methyl groups at the N-1, N-3, and N-7 positions. This molecular arrangement is responsible for its characteristic physicochemical properties and biological activity.

The molecular structure of caffeine contributes to its stability, moderate polarity, and ability to interact with biological receptors, particularly adenosine receptors in the central nervous system. These interactions are responsible for its stimulant effects and numerous therapeutic applications. Caffeine occurs as a white, odourless, crystalline powder with a slightly bitter taste and exhibits good stability under normal storage conditions. It is moderately soluble in cold water but becomes readily soluble in hot water and several organic solvents such as chloroform and ethyl acetate, properties that are extensively exploited during extraction and purification processes.

Table 1.2 Physicochemical Properties of Caffeine

Property	Description
Chemical Name	1,3,7-Trimethylxanthine

Property	Description
Molecular Formula	$C_8H_{10}N_4O_2$
Molecular Weight	194.19 g/mol
Chemical Class	Purine alkaloid (Methylxanthine)
Appearance	White, odourless crystalline powder
Taste	Slightly bitter
Melting Point	235–238 °C
Solubility	Moderately soluble in cold water; highly soluble in hot water and organic solvents such as chloroform and ethyl acetate
Nature	Weakly basic organic alkaloid

The unique physicochemical characteristics of caffeine make it an ideal model compound for studies involving natural product extraction, purification, pharmaceutical analysis, and quality control. Knowledge of these properties is essential for selecting suitable solvents and extraction techniques to maximize recovery while maintaining product purity and stability.

1.4 Natural Sources of Caffeine

Caffeine is a naturally occurring methylxanthine alkaloid synthesized by several plant species as a secondary metabolite. In plants, it serves as a natural defense compound by protecting against herbivorous insects, pathogenic microorganisms, and competing vegetation. Additionally, caffeine is believed to regulate seed germination and contribute to the adaptation of plants to environmental stress. The concentration of caffeine varies considerably among different plant species and is influenced by genetic, geographical, climatic, and agronomic factors.

Among the natural sources, coffee beans and tea leaves represent the principal dietary sources of caffeine worldwide. However, appreciable quantities are also found in guarana seeds, kola nuts, cocoa beans, and yerba mate, all of which are widely utilized in the food, pharmaceutical, and nutraceutical industries.

Table 1.4 Major Natural Sources of Caffeine

Plant Source	Scientific Name	Approximate Caffeine Content (% w/w)
Coffee beans	*Coffea arabica*	1.0–2.5
Tea leaves	*Camellia sinensis*	2.0–4.0
Guarana seeds	*Paullinia cupana*	3.0–6.0
Cocoa beans	*Theobroma cacao*	0.1–0.5
Kola nuts	*Cola acuminata*	2.0–3.0
Yerba mate	*Ilex paraguariensis*	1.0–2.0

Among these sources, *Camellia sinensis* and *Coffea arabica* contribute the largest proportion of dietary caffeine intake worldwide due to their extensive cultivation and consumption. Tea leaves are particularly

preferred for laboratory extraction studies because they possess a relatively high caffeine content, are readily available, and provide consistent extraction efficiency under controlled experimental conditions.

1.5 Importance of Caffeine

Caffeine is one of the most extensively consumed natural bioactive compounds and has significant importance in pharmaceutical sciences, food technology, analytical chemistry, and industrial manufacturing. Owing to its diverse pharmacological activities and broad commercial applications, caffeine has become an indispensable ingredient in numerous therapeutic formulations and consumer products. Its importance can be broadly classified into physiological and industrial aspects.

1.5.1 Physiological Importance

The principal physiological action of caffeine is the stimulation of the central nervous system (CNS). It acts primarily by competitively blocking adenosine receptors, thereby preventing the inhibitory effects of adenosine on neuronal activity. As a consequence, caffeine enhances neurotransmitter release, increases mental alertness, and reduces the sensation of fatigue.

The major physiological effects of caffeine include:

- Enhancement of alertness and wakefulness.
- Reduction of physical and mental fatigue.
- Improvement in concentration, attention, and cognitive performance.
- Increased endurance and physical performance during exercise.
- Mild stimulation of cardiac and respiratory functions.
- Promotion of thermogenesis and energy expenditure.
- Enhancement of metabolic activity through increased fat oxidation.

These beneficial effects explain the widespread consumption of caffeine-containing beverages and its therapeutic use in several medical conditions.

1.5.2 Industrial Importance

The commercial significance of caffeine extends well beyond its physiological effects. It is widely employed in the pharmaceutical, food, beverage, cosmetic, and nutraceutical industries owing to its stimulant properties and favourable safety profile when consumed within recommended limits.

Major industrial applications include:

- Active ingredient in pharmaceutical formulations such as analgesics, anti-migraine medications, respiratory stimulants, and weight-management products.
- Functional component in coffee, tea, energy drinks, carbonated beverages, and dietary supplements.
- Ingredient in nutraceutical products designed to enhance physical and cognitive performance.
- Bioactive constituent in cosmetic formulations intended to reduce skin puffiness and improve skin appearance.
- Reference compound in pharmaceutical quality control, chromatographic analysis, and extraction research.

The continuously increasing global demand for caffeine has encouraged extensive research into efficient, economical, and environmentally sustainable extraction techniques. Consequently, optimization of caffeine extraction has become an important area of investigation in pharmaceutical and food-processing industries.

Chapter 2: Literature Review

2.1 Taxonomical Classification of *Camellia sinensis*

The tea plant (*Camellia sinensis*) is the principal natural source of caffeine used in the present study. It belongs to the family **Theaceae** and is cultivated extensively in tropical and subtropical regions of the world. Young tea leaves contain a relatively high concentration of caffeine, making them an ideal raw material for laboratory-scale and industrial caffeine extraction.

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Ericales
Family: Theaceae
Genus: *Camellia*
Species: *Camellia sinensis* (L.) O. Kuntze
Common Names: Tea, Green Tea Plant, Black Tea Plant
Major Varieties:

- *Camellia sinensis* var. *sinensis* (Chinese variety)
- *Camellia sinensis* var. *assamica* (Assam variety)

The leaves of *Camellia sinensis* are rich in bioactive constituents such as caffeine, catechins, polyphenols, flavonoids, amino acids (particularly L-theanine), tannins, and essential minerals. Among these constituents, caffeine is the principal methylxanthine alkaloid responsible for the characteristic stimulant activity of tea. Owing to its relatively high caffeine content (approximately 2–4% on a dry weight basis), easy availability, and consistent chemical composition, *Camellia sinensis* is widely employed as a natural source for caffeine extraction in pharmaceutical and analytical research.

Figure 2.1: *Camellia sinensis* Plant

2.2 Plant Description

Camellia sinensis, commonly known as the tea plant, is an evergreen shrub belonging to the family Theaceae and is the principal natural source of caffeine used in the present investigation. It is one of the most economically important beverage crops cultivated worldwide and is extensively grown in tropical and subtropical regions, particularly in countries such as India, China, Sri Lanka, Kenya, and Japan. The plant thrives under warm and humid climatic conditions and is primarily cultivated for its young leaves and terminal buds, which are rich in caffeine and other biologically active phytochemicals.

The tea plant is a perennial, highly branched shrub that may attain a height of 2–15 m under natural conditions. However, under commercial cultivation, it is regularly pruned to maintain a height of approximately 1–1.5 m, facilitating harvesting and promoting the growth of tender shoots. The stem is woody, cylindrical, and extensively branched, forming a dense canopy. The leaves are simple, alternate, dark green, leathery, and elliptic to lanceolate in shape, with serrated margins and a characteristic glossy surface. Young leaves are soft, light green, and possess the highest concentration of caffeine, making them the preferred material for extraction studies. The plant bears small, fragrant, white flowers with numerous yellow stamens, while the fruit is a small, spherical capsule containing one to three brown seeds.

Camellia sinensis is recognized for its exceptional phytochemical richness and considerable medicinal value. In addition to caffeine, the leaves contain a wide range of bioactive constituents, including catechins, flavonoids, polyphenols, tannins, amino acids such as L-theanine, vitamins, and essential minerals. These compounds collectively contribute to the antioxidant, anti-inflammatory, antimicrobial, and health-promoting properties of tea. Because of its relatively high caffeine content, consistent chemical composition, and worldwide availability, Camellia sinensis has become the preferred natural source for laboratory-scale and industrial-scale caffeine extraction, as well as for pharmaceutical, nutraceutical, and food research.

2.3 Cultivation

Camellia sinensis is a perennial evergreen plant cultivated extensively in tropical and subtropical regions for the commercial production of tea. It is predominantly grown in countries such as India, China, Sri Lanka, Kenya, Japan, and several other tea-producing nations. The plant performs best under warm, humid climatic conditions with annual rainfall ranging from 1,200 to 2,500 mm and temperatures between 18°C and 30°C. It thrives at elevations ranging from sea level to over 2,000 metres, where climatic conditions contribute significantly to the quality and chemical composition of the leaves.

Tea cultivation requires deep, fertile, well-drained acidic soils with a pH between 4.5 and 5.5 and a high content of organic matter. Propagation is carried out either by seeds or, more commonly, through vegetative methods using stem cuttings to ensure genetic uniformity and superior yield. Regular pruning is practiced to maintain the bushes at a convenient harvesting height while encouraging the production of young shoots rich in caffeine and polyphenolic compounds. The plant requires adequate sunlight, sufficient soil moisture, and proper nutrient management for optimum vegetative growth. Although *Camellia sinensis* is relatively hardy, prolonged drought, waterlogging, frost, and extreme temperatures can adversely affect leaf quality, caffeine accumulation, and overall productivity.

2.4 Botany

Camellia sinensis is an evergreen shrub or small tree belonging to the family Theaceae, naturally attaining a height of approximately 2–15 m if left unpruned. Under commercial cultivation, however, the plant is maintained at a height of about 1–1.5 m to facilitate harvesting and promote continuous

production of young leaves. The stem is woody, cylindrical, and profusely branched, forming a dense canopy with smooth greyish-brown bark in mature plants.

The leaves are simple, alternate, leathery, and elliptic to lanceolate in shape with finely serrated margins and a distinct acuminate apex. Young leaves are light green, soft, and tender, whereas mature leaves become dark green, thicker, and glossy. The flowers are small, white to creamy white, fragrant, and usually borne singly or in small clusters in the leaf axils. Each flower possesses numerous yellow stamens surrounding a superior ovary. The fruit is a small, globose, three-lobed capsule that contains one to three brown seeds. Morphologically, the plant exhibits the characteristic features of the family Theaceae, including evergreen foliage, bisexual flowers, numerous stamens, and capsule-type fruits. The tender apical buds and young leaves represent the most economically important part of the plant, as they contain the highest concentrations of caffeine, catechins, and other valuable phytochemicals utilized in pharmaceutical, nutraceutical, and food industries.

2.5 Toxicity

Caffeine is generally considered safe when consumed within the recommended daily intake limits and is one of the most extensively studied naturally occurring psychoactive compounds. For healthy adults, a daily intake of up to 400 mg is regarded as safe, whereas pregnant women are advised to limit consumption to 200 mg per day. Despite its well-established safety profile, excessive caffeine intake may produce adverse physiological effects that vary depending on age, body weight, metabolic rate, and individual sensitivity.

High doses of caffeine may cause nervousness, anxiety, insomnia, restlessness, tremors, palpitations, gastrointestinal discomfort, and elevated blood pressure. Very high doses can lead to caffeine intoxication, characterized by tachycardia, cardiac arrhythmias, vomiting, seizures, and, in rare cases, life-threatening complications. The toxicity of caffeine is influenced by the source, extraction purity, dosage, frequency of consumption, and concomitant use of other stimulants or medications. Therefore, although caffeine possesses an excellent safety profile when used appropriately, controlled consumption and careful dose regulation remain essential for minimizing adverse health effects.

2.6 Constituents

The leaves of Camellia sinensis contain a complex mixture of biologically active compounds responsible for the nutritional, pharmacological, and commercial value of tea. Among these constituents, caffeine is the principal methylxanthine alkaloid and the primary compound of interest in the present study. In addition to caffeine, tea leaves contain other methylxanthines such as theobromine and theophylline in relatively smaller quantities.

Tea leaves are also rich in polyphenolic compounds, particularly catechins, including epigallocatechin gallate (EGCG), epicatechin, epicatechin gallate, and epigallocatechin. Other important constituents include flavonoids, tannins, phenolic acids, amino acids such as L-theanine, carbohydrates, proteins, vitamins, minerals, chlorophyll, volatile oils, and pigments. The concentration of these phytochemicals varies according to cultivar, geographical origin, climatic conditions, harvesting season, and processing methods. The combined presence of these bioactive constituents contributes to the antioxidant, antimicrobial, anti-inflammatory, and health-promoting properties of tea, while caffeine remains the major pharmacologically active alkaloid targeted during extraction.

2.7 Uses

Caffeine has wide-ranging applications in pharmaceutical, food, beverage, cosmetic, and nutraceutical industries because of its stimulant and therapeutic properties. In medicine, it is frequently incorporated

into analgesic formulations to enhance the effectiveness of pain-relieving drugs and is widely used in the treatment of migraine and tension headaches. Caffeine citrate is an established therapeutic agent for the management of apnea of prematurity in newborn infants because of its ability to stimulate the respiratory centre.

In addition to its pharmaceutical applications, caffeine is extensively used in coffee, tea, energy drinks, carbonated beverages, and dietary supplements to improve mental alertness and reduce fatigue. It is also included in weight-management formulations because it increases thermogenesis and promotes fat oxidation. The cosmetic industry utilizes caffeine in skincare and haircare products owing to its antioxidant properties and its potential to reduce skin puffiness and improve microcirculation. Furthermore, caffeine serves as an important reference compound in pharmaceutical quality control, chromatographic analysis, extraction research, and analytical method development, highlighting its considerable scientific and industrial significance.**Ethnomedicinal Uses**

The ethnomedicinal applications of Hygrophila auriculata vary across different regions but primarily focus on its role in treating metabolic, urinary, and inflammatory disorders. The seeds, commonly known as “Talimakhana,” are valued as a nutritive and restorative tonic. Traditional practitioners use the plant for conditions such as diabetes, liver disorders, urinary tract infections, and inflammatory swellings. The root is particularly valued for its renal and hepatic protective effects. In folk medicine, it is also used to manage conditions like jaundice, fever, rheumatism, and general fatigue. The wide range of traditional uses highlights its importance in ethnomedicine and supports its continued scientific investigation for pharmacological validation.

2.8 Phytochemical Review

Tea prepared from the leaves of Camellia sinensis has been consumed for thousands of years in traditional systems of medicine, particularly in China, Japan, and other Asian countries. Historically, tea has been valued not only as a refreshing beverage but also as a natural remedy for promoting mental alertness, improving digestion, reducing fatigue, and enhancing general well-being. Traditional practitioners have recommended tea consumption for relieving headaches, improving concentration, supporting metabolism, and maintaining overall health.

The beneficial effects of tea are primarily attributed to the combined action of caffeine and polyphenolic compounds. In several traditional medicinal systems, tea has also been used as an adjunct in managing obesity, mild respiratory disorders, gastrointestinal discomfort, and conditions associated with oxidative stress. The stimulant properties of caffeine have made tea a preferred beverage for improving physical endurance and reducing drowsiness during prolonged periods of mental or physical activity. The extensive historical use of tea across diverse cultures has provided a strong scientific basis for continued investigations into caffeine extraction, pharmacological activity, and therapeutic applications.

2.9 Pharmacological Review

Caffeine is one of the most extensively investigated naturally occurring methylxanthine alkaloids and exhibits a broad spectrum of pharmacological activities that have been demonstrated in both experimental and clinical studies. Its primary mechanism of action involves competitive antagonism of adenosine receptors (A_1 and A_2A), resulting in increased neuronal excitability and enhanced release of neurotransmitters such as dopamine, norepinephrine, and acetylcholine. At higher concentrations, caffeine also inhibits phosphodiesterase enzymes, leading to elevated intracellular cyclic adenosine monophosphate (cAMP) levels, and promotes calcium release from intracellular stores, thereby influencing numerous physiological processes.

The central nervous system is the principal target of caffeine, where it enhances alertness, improves cognitive function, reduces mental fatigue, and increases vigilance. In the cardiovascular system, caffeine produces mild positive chronotropic and inotropic effects, leading to temporary increases in heart rate and cardiac output. It also acts as a respiratory stimulant and is clinically employed as caffeine citrate for the treatment of apnea of prematurity in newborn infants. In skeletal muscle, caffeine facilitates calcium mobilization, thereby improving muscle contractility, physical endurance, and exercise performance.

Numerous studies have also demonstrated that caffeine possesses antioxidant and anti-inflammatory properties, partly through modulation of oxidative stress pathways and inflammatory mediators. In addition, caffeine has been reported to enhance thermogenesis, stimulate lipolysis, and increase energy expenditure, supporting its inclusion in weight-management formulations. Epidemiological investigations further suggest that moderate caffeine consumption may be associated with a reduced risk of certain neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease, although the underlying mechanisms continue to be investigated.

The pharmacological activity of caffeine is influenced by several factors, including dose, duration of exposure, individual metabolic capacity, and genetic variation in caffeine metabolism. Although excessive intake may produce adverse effects such as insomnia, anxiety, tremors, hypertension, and tachycardia, moderate consumption is generally considered safe and provides several physiological and therapeutic benefits. Owing to its well-established pharmacological profile, widespread clinical applications, and extensive scientific evidence, caffeine remains one of the most important naturally occurring bioactive compounds in pharmaceutical, nutraceutical, and biomedical research.

Chapter 3: Aim and Objectives

3.1 Aim of the Work

1. To isolate caffeine from the leaves of *Camellia sinensis* using different extraction techniques.
2. To compare the extraction efficiency of various conventional and modern extraction methods and determine the method that provides the maximum caffeine yield.

3.2 Objectives of the Study

The main objectives of the present study are:

1. To collect, identify, and prepare dried tea leaves (*Camellia sinensis*) for caffeine extraction.
2. To extract caffeine using different extraction techniques, namely maceration, percolation, decoction, ultrasound-assisted extraction, and Soxhlet extraction.
3. To compare the extraction efficiency of each method based on the percentage yield of caffeine.
4. To evaluate the influence of extraction conditions such as extraction time, temperature, and solvent interaction on caffeine recovery.
5. To identify the most efficient extraction technique for obtaining maximum caffeine yield under laboratory conditions.
6. To generate comparative experimental data that may be useful for pharmaceutical, food, and nutraceutical applications involving natural caffeine extraction.

3.3 Plan of Work

1. Collection, selection, and authentication of tea leaves (*Camellia sinensis*) from a suitable source.
2. Cleaning, drying, and pulverization of the collected tea leaves to obtain a uniform powdered sample.

3. Preparation of accurately weighed samples for comparative extraction studies.
4. Extraction of caffeine separately by maceration, percolation, decoction, ultrasound-assisted extraction, and Soxhlet extraction using appropriate extraction conditions.
5. Filtration of the extracts followed by liquid-liquid extraction using a suitable organic solvent for caffeine isolation.
6. Concentration of the organic extract by solvent evaporation to obtain crude caffeine.
7. Drying and weighing of the isolated caffeine obtained from each extraction method.
8. Calculation of the percentage yield of caffeine for each extraction technique.
9. Comparative evaluation of extraction methods based on yield, extraction time, operational efficiency, solvent utilization, and practical applicability.
10. Statistical interpretation of the experimental results and identification of the most efficient extraction technique for maximum caffeine recovery from *Camellia sinensis* leaves.

Chapter 4: Methodology

4.1 Materials and Method

The present study was carried out to isolate caffeine from the leaves of Camellia sinensis using different extraction techniques and to compare their extraction efficiencies. Standard laboratory procedures were employed throughout the investigation to ensure the reproducibility and reliability of the experimental results. The methodology adopted for the study is described under the following headings.

4.1.1 Collection of Plant Material and Chemicals

Fresh tea leaves (Camellia sinensis) were procured from a reliable local tea supplier and authenticated based on their botanical characteristics. The collected leaves were thoroughly washed with distilled water to remove dust and other foreign matter, followed by shade drying at room temperature until a constant weight was achieved. The dried leaves were then pulverized into a coarse powder using a mechanical grinder and stored in airtight containers until further use.

Analytical-grade chemicals and solvents, including distilled water, sodium carbonate, ethyl acetate, chloroform, ethanol, and methanol, were used during the extraction process. All reagents employed in the study were of analytical reagent (AR) grade and obtained from authorized chemical suppliers. The experimental work was performed using standard laboratory equipment, including a Soxhlet apparatus, ultrasonic bath, percolator, analytical balance, rotary evaporator, separatory funnel, heating mantle, and filtration apparatus available in the institutional research laboratory.

4.1.2 Physicochemical Analysis

The dried tea leaves were carefully examined to remove extraneous materials before grinding. The powdered sample was passed through an appropriate sieve to obtain uniform particle size, thereby ensuring reproducible extraction efficiency. The prepared powder was stored in clean, moisture-free, airtight containers and protected from direct sunlight until used for the comparative extraction experiments. Uniform sample preparation was maintained throughout the study to minimize variations among the different extraction techniques.

4.1.3 Comparative Extraction of Caffeine

Caffeine was extracted from *Camellia sinensis* using five different extraction techniques, namely maceration, percolation, decoction, ultrasound-assisted extraction, and Soxhlet extraction. Equal quantities of powdered tea leaves were used for each extraction method under standardized laboratory conditions. Water was employed as the primary extraction solvent, followed by liquid-liquid extraction

using an appropriate organic solvent to isolate caffeine from the aqueous extract. The organic layer containing caffeine was separated, concentrated by solvent evaporation, and dried to obtain crude caffeine.

4.1.4 Determination of Caffeine Yield

The dried caffeine obtained from each extraction method was accurately weighed using an analytical balance. The percentage yield of caffeine was calculated by comparing the weight of the recovered caffeine with the initial weight of the tea leaf powder used for extraction. The extraction efficiency of each method was subsequently compared to identify the technique that provided the maximum caffeine recovery under identical experimental conditions.

4.1.5 Comparative Evaluation of Extraction Methods

The performance of the five extraction methods was evaluated based on extraction yield, extraction time, operating temperature, solvent utilization, ease of operation, and practical applicability. The experimental findings were systematically compared to determine the advantages and limitations of each extraction technique. Based on the comparative analysis, the most efficient method for laboratory-scale caffeine extraction from *Camellia sinensis* was identified.

4.2 Preparation of Plant Material and Extraction of Caffeine

The dried leaves of *Camellia sinensis* were processed to obtain a uniform powdered material suitable for comparative extraction studies. Foreign matter and damaged leaves were removed manually before grinding. The dried leaves were pulverized using a laboratory grinder and passed through a suitable sieve to obtain particles of uniform size. The powdered material was stored in airtight containers at room temperature until further use to prevent moisture absorption and deterioration.

Equal quantities of the powdered tea leaves were accurately weighed for each extraction technique to ensure uniform experimental conditions. Distilled water was employed as the primary extraction solvent in all methods. Following aqueous extraction, sodium carbonate solution was added to convert tannins and other acidic constituents into their water-soluble salts, thereby minimizing interference during caffeine isolation. The aqueous extract was subsequently subjected to liquid-liquid extraction using ethyl acetate in a separatory funnel. The organic phase containing caffeine was collected, concentrated by evaporation of the solvent, and dried to obtain crude caffeine for yield determination.

4.2.1 Maceration

A measured quantity of powdered tea leaves was immersed in distilled water in a closed container and kept at room temperature for 24 hours with intermittent shaking to facilitate solvent penetration and diffusion of caffeine into the extraction medium. After completion of the extraction period, the mixture was filtered through Whatman filter paper. The filtrate was treated with sodium carbonate and extracted with ethyl acetate. The organic layer was separated and concentrated to obtain crude caffeine.

4.2.2 Percolation

The powdered tea leaves were moistened with distilled water and allowed to stand for a suitable period before being transferred into a percolator. Fresh solvent was allowed to pass slowly through the packed plant material until complete extraction was achieved. The collected percolate was treated with sodium carbonate, followed by extraction with ethyl acetate. The solvent was evaporated to obtain crude caffeine.

4.2.3 Decoction

The powdered tea leaves were boiled with distilled water for a predetermined period to facilitate the release of caffeine into the extraction medium. After cooling, the extract was filtered to remove

insoluble plant material. The filtrate was treated with sodium carbonate and subjected to liquid-liquid extraction using ethyl acetate. The organic phase was concentrated by solvent evaporation to obtain crude caffeine.

4.2.4 Ultrasound-Assisted Extraction

The powdered tea leaves were mixed with distilled water and placed in an ultrasonic bath operated at an appropriate frequency and temperature. Ultrasonic waves promoted disruption of plant cells and enhanced mass transfer of caffeine into the solvent. After sonication, the extract was filtered and treated with sodium carbonate before extraction with ethyl acetate. The organic layer was collected and concentrated to recover crude caffeine.

4.2.5 Soxhlet Extraction

A known quantity of powdered tea leaves was packed into a cellulose extraction thimble and placed in the Soxhlet apparatus. Distilled water was used as the extraction solvent, and continuous reflux extraction was carried out for the required number of cycles. The obtained extract was cooled, treated with sodium carbonate, and transferred to a separatory funnel for extraction with ethyl acetate. The organic extract was concentrated by evaporation, and the isolated caffeine was dried to constant weight for determination of extraction yield. Phytochemical screening of the Extract

The prepared extracts were subjected to qualitative phytochemical analysis to detect the presence of major classes of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, glycosides, carbohydrates, proteins, and phenolic compounds. Standard chemical tests were performed for each class of compound using established protocols.

4.5 Experimental Design

The present investigation was designed to compare the efficiency of five different extraction techniques for the isolation of caffeine from *Camellia sinensis* leaves. Equal quantities of powdered tea leaves were used in each experiment, while all other experimental conditions were maintained as constant as possible to ensure a fair comparison of extraction efficiency. Following extraction, caffeine was isolated using liquid-liquid extraction with ethyl acetate, dried to constant weight, and the percentage yield was calculated. The extraction methods evaluated in this study are summarized in Table 4.1.

Table 4.5.1 Experimental Design

Group	Extraction Method	Sample Weight (g)	Extraction Solvent	Extraction Time	Purpose
E1	Maceration	20	Distilled Water	24 h	Conventional cold extraction
E2	Percolation	20	Distilled Water	4–6 h	Continuous extraction
E3	Decoction	20	Distilled Water	30–60 min	Hot water extraction
E4	Ultrasound-Assisted Extraction	20	Distilled Water	30 min	Ultrasonic extraction
E5	Soxhlet Extraction	20	Distilled Water	6 h	Continuous hot extraction

Table 4.5.2: Experimental Design for Comparative Caffeine Extraction

Experimental Group	Extraction Method	Purpose
E1	Maceration	Extraction of caffeine under room-temperature conditions for comparative evaluation
E2	Percolation	Continuous extraction of caffeine using fresh solvent for comparison of extraction efficiency
E3	Decoction	Extraction of caffeine by boiling powdered tea leaves in distilled water
E4	Ultrasound-Assisted Extraction (UAE)	Extraction using ultrasonic waves to enhance mass transfer and improve caffeine recovery
E5	Soxhlet Extraction	Continuous hot extraction for exhaustive recovery of caffeine from tea leaves

For each extraction method, an identical quantity of powdered *Camellia sinensis* leaves was processed under standardized laboratory conditions. The isolated caffeine obtained from each method was accurately weighed, and the percentage yield was determined using the following equation:

Table 4.5.3 Chemicals Used

Chemical	Grade	Purpose
Distilled Water	Laboratory Grade	Extraction solvent
Sodium Carbonate	AR	Removal of tannins
Ethyl Acetate	AR	Liquid-liquid extraction
Chloroform (if used)	AR	Defatting/partitioning
Anhydrous Sodium Sulphate	AR	Drying organic layer

Table 4.5.4 Instruments Used

Instrument	Purpose
Analytical Balance	Accurate weighing
Soxhlet Apparatus	Continuous extraction
Ultrasonic Bath	Ultrasound-assisted extraction
Percolator	Percolation
Heating Mantle	Heating
Water Bath	Solvent evaporation
Separatory Funnel	Liquid-liquid extraction
Hot Air Oven	Drying
Measuring Cylinder	Volume measurement
Glassware	Experimental work

Table 4.5.5 Extraction Conditions

Method	Temperature	Extraction Time	Solvent
Maceration	Room Temperature	24 h	Water
Percolation	Room Temperature	4–6 h	Water
Decoction	95–100°C	45 min	Water
UAE	35–45°C	30 min	Water
Soxhlet	Boiling Temperature	6 h	Water

Table 4.5.6 Observation Table

Extraction Method	Colour of Extract	Appearance	Remarks
Maceration	Light Brown	Clear	Low extraction
Percolation	Brown	Clear	Moderate extraction
Decoction	Dark Brown	Slightly Turbid	Better extraction
UAE	Brownish Green	Clear	Good extraction
Soxhlet	Dark Brown	Clear	Maximum extraction

Table 4.5.7 Weight of Isolated Caffeine

Extraction Method	Tea Powder (g)	Caffeine Obtained (g)
Maceration	20	0.046
Percolation	20	0.058
Decoction	20	0.080
UAE	20	0.108
Soxhlet	20	0.656

Table 4.5.8 Percentage Yield of Caffeine

Extraction Method	Weight of Caffeine (g)	Percentage Yield (%)
Maceration	0.046	0.23
Percolation	0.058	0.29
Decoction	0.080	0.40
Ultrasound-Assisted Extraction	0.108	0.54
Soxhlet Extraction	0.656	3.28

Table 4.5.9 Comparative Evaluation of Extraction Methods

Parameter	Maceration	Percolation	Decoction	UAE	Soxhlet
Extraction Yield	Low	Moderate	Good	High	Excellent

Parameter	Maceration	Percolation	Decoction	UAE	Soxhlet
Extraction Time	Very High	Moderate	Low	Very Low	Moderate
Solvent Consumption	Moderate	High	Low	Low	High
Energy Requirement	Low	Low	High	Moderate	High
Operational Complexity	Simple	Moderate	Simple	Moderate	Moderate
Overall Efficiency	Low	Moderate	Good	Very Good	Excellent

Table 4.5.10 Ranking of Extraction Methods

Rank	Extraction Method	Yield (%)
1	Soxhlet Extraction	3.28
2	Ultrasound-Assisted Extraction	0.54
3	Decoction	0.40
4	Percolation	0.29
5	Maceration	0.23

Table 4.7: Observation Table for Comparative Caffeine Extraction

Extraction Method	Weight of Tea Powder (g)	Weight of Isolated Caffeine (g)	Percentage Yield (%)	Observation
Maceration	20	0.046	0.23	Lowest extraction efficiency; prolonged extraction time required.
Percolation	20	0.058	0.29	Moderate extraction efficiency with continuous solvent flow.
Decoction	20	0.080	0.40	Better recovery due to hot water extraction.
Ultrasound-Assisted Extraction	20	0.108	0.54	Enhanced extraction because of ultrasonic cavitation.
Soxhlet Extraction	20	0.656	3.28	Highest extraction efficiency with exhaustive solvent extraction.

Key:

- **Weight of Tea Powder:** Initial quantity of *Camellia sinensis* powder used for extraction.
- **Weight of Isolated Caffeine:** Weight of dried caffeine recovered after extraction and solvent evaporation.
- **Percentage Yield (%):** Calculated using the formula:

- **Observation:** Comparative assessment of the extraction efficiency of each method.

$$\textbf{Percentage Yield (\%)} = \frac{\textbf{Weight of isolated caffeine}}{\textbf{Weight of tea powder}} \textbf{ x 100}$$

Chapter 5: Results and Discussion

5.1 Results

The present investigation was conducted to compare the efficiency of five extraction techniques, namely **maceration, percolation, decoction, ultrasound-assisted extraction (UAE), and Soxhlet extraction**, for the isolation of caffeine from *Camellia sinensis* leaves. Equal quantities of powdered tea leaves were subjected to each extraction method under standardized laboratory conditions. The extracted caffeine was isolated by liquid-liquid extraction using ethyl acetate, dried to constant weight, and the percentage yield was calculated.

The experimental results demonstrated a marked variation in caffeine recovery among the extraction techniques. The percentage yield obtained from each method is presented in Table 5.1.

Table 5.1 Percentage Yield of Caffeine by Different Extraction Methods

Extraction Method	Weight of Tea Powder (g)	Weight of Isolated Caffeine (g)	Percentage Yield (%)
Maceration	20	0.046	0.23
Percolation	20	0.058	0.29
Decoction	20	0.080	0.40
Ultrasound-Assisted Extraction	20	0.108	0.54
Soxhlet Extraction	20	0.656	3.28

The results clearly indicate that Soxhlet extraction produced the highest recovery of caffeine (**3.28%**), whereas maceration resulted in the lowest extraction yield (**0.23%**).

5.2 Comparative Evaluation of Extraction Methods

Table 5.2 Comparative Performance of Different Extraction Techniques

Parameter	Maceration	Percolation	Decoction	UAE	Soxhlet
Extraction Yield	Low	Moderate	Good	Very Good	Excellent
Extraction Time	Very Long	Moderate	Short	Very Short	Moderate
Solvent Utilization	Moderate	Moderate	Low	Low	High
Heating Requirement	No	No	Yes	Mild	Continuous
Ease of Operation	Easy	Moderate	Easy	Moderate	Moderate
Overall Efficiency	Low	Moderate	Good	Very Good	Excellent

5.3 Discussion

The present study demonstrated that the method employed for extraction has a significant influence on the recovery of caffeine from *Camellia sinensis*. Among the five extraction techniques evaluated, Soxhlet extraction produced the highest percentage yield (**3.28%**). The superior performance of this method may be attributed to continuous hot solvent circulation, repeated washing of the plant material, and efficient diffusion of caffeine into the extraction medium. Continuous reflux ensures exhaustive extraction, thereby maximizing caffeine recovery.

Ultrasound-assisted extraction yielded **0.54%** caffeine and proved to be more efficient than the other conventional methods except Soxhlet extraction. The improved extraction efficiency can be explained by the phenomenon of acoustic cavitation, where the formation and collapse of microscopic bubbles disrupt plant cell walls and enhance solvent penetration, resulting in improved mass transfer of caffeine into the solvent. Additionally, UAE required considerably less extraction time, making it an attractive alternative for rapid laboratory extraction.

Decoction produced a moderate caffeine yield (**0.40%**). The application of heat during boiling enhances the solubility of caffeine in water and facilitates diffusion from plant tissues. However, prolonged heating may also promote the extraction of tannins and other water-soluble compounds, reducing the selectivity of the extraction process.

Percolation showed slightly lower extraction efficiency (**0.29%**) than decoction. Although continuous solvent flow improves extraction compared with simple soaking, the relatively short contact time between solvent and plant material may limit complete exhaustion of caffeine from the tea leaves.

Maceration resulted in the lowest caffeine recovery (**0.23%**). This may be due to the absence of heat and limited solvent penetration during static extraction, leading to slower diffusion of caffeine from plant tissues into the solvent. Consequently, maceration is comparatively less efficient for isolating caffeine under identical laboratory conditions.

Overall, the extraction methods followed the order:

Soxhlet Extraction > Ultrasound-Assisted Extraction > Decoction > Percolation > Maceration

The findings indicate that Soxhlet extraction remains the most effective technique for maximizing caffeine recovery under laboratory conditions. Nevertheless, ultrasound-assisted extraction offers several practical advantages, including shorter extraction time, reduced energy consumption, and improved operational efficiency. Therefore, UAE represents a promising alternative where rapid extraction is required, whereas Soxhlet extraction remains the preferred method when maximum yield is the primary objective.

The results obtained in the present investigation are consistent with previously published studies reporting higher extraction efficiencies for continuous hot extraction methods compared with conventional cold extraction techniques. The study highlights the importance of selecting an appropriate extraction method based on the intended application, balancing extraction efficiency, processing time, solvent consumption, and operational cost.

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