

Sudan University of Science and Technology

College of Science

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EXTRACTION OF CATECHIN  
COMPOUNDS FROM GREEN TEA AND  
STUDY THEIR EFFECT ON BACTERIA

A Thesis Submitted in Partial Fulfillment for the  
Degree of B.Sc (HON)

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بسم الله الرحمن الرحيم

قال تعالى:

(( أمن هو قانت ءاناء الليل ساجدا وقائما يحذر الأخرة ويرجوا  
رحمة ربه قل هل يستوى الذين يعلمون والذين لا يعلمون انما  
يتذكر أولوا الألباب))

سورة الزمر 9

# الاهداء

الي من علمني العطاء دون انتظار..الي من أحمل اسمه بكل افتخار.. الي من كان شمعة  
متقدة تنير ظلمة حياتي..ستبقى كلماتك نجوم اهتدي بها اليوم وفي الغد  
والي الأبد..وستظل ذكراك حية في قلبي...والذي الحبيب

الي ملاكي في الحياة..الي نبع الحنان..الي بسمه الحياة..الي من كان دعائها سر نجاحي..الي  
ست الحبايب..أمي الحبيبة

الي من عرفت معها معني الحياة...الي من بها أكبر وعلينا أعتمد..الي من تطلعت لنجاحي  
بنظرات الأمل.. أختي الغالية

الي من بوجودهم أكتسب القوة والمحبة....اخوتي الاعزاء

الي من تميزوا بالوفاء..الي من معهم سعدت, وبرفقتهم في دروب الحياة الحلوة والحزينة  
سرت الي من كانوا معي علي طريق النجاح..الي من عرفت كيف أجدهم وعلموني أن لا  
أضيعهم.

..صديقاتي..

# *Acknowledgement*

**I wish to express my deepest gratitude to my supervisor D. Amani Abdulrazig for her appreciable help, advice and for her encouragement since the start of this project.**

# Abstract

This study explains the extraction of catechin compounds from Cofftea green tea as potential sources of antibacterial materials. The methodologies of solvent extraction and partition were utilized to recover catechin compounds from green tea. The optimum experimental condition was obtained by optimizing operating factors. After extracting the green tea with water at 80 C for 40 min, the extract was partitioned with water/chloroform, which was best suited to remove caffeine impurity from the extract. Further, the resulting extract was partitioned water/ethyl acetate to deeply purify the catechin compounds of EGC, EC, EGCG and ECG. The present study showed that the total content of catechin compounds in cofftea green tea is 3.90%.

Recent research indicates that green tea has numerous health benefits, including the ability to kill bacteria. This lab test the antimicrobial properties of green tea (with and without table salt) against *E.coli* and *S.aureus* in paper disk diffusion. The results indicated that the inhibition zone diameter of GTE for *E.coli* is 18 mm. And 20 mm when we add salt. *S.aureus* showed no zone of inhibition. The lack of antimicrobial properties for the tea sample against *S.aureus* may has been due to preparation method. But it showed inhibition zone with 12 mm when we add salt.

This experiment showed that the antimicrobial properties increase with adding table salt.

# المستخلص

هذه الدراسة تشرح استخلاص الكاتشينات من شاي كوفتي الاخضر كمصدر للمواد المضادة للبكتيريا. وفي هذه الدراسة استخدمت طريقة الاستخلاص بالمذيب مع التحكم بالعوامل المؤثرة. تم استخلاص الكاتشينات بواسطة الماء عند درجة حرارة 80 درجة مئوية لمدة 40 دقيقة. واستخدمت مادة الكلوروفورم للتخلص من الكافيين والشوائب الأخرى باستخدام قمع الفصل بعد إزالة الشوائب تم استخدام خلاص الايثيل للحصول علي كاتشينات نقية. وبينت الدراسة ان شاي كوفتي يحتوي علي %3.90 من الكاتشينات.

الابحاث في الالونة الاخيرة حددت عدد من فوائد الشاي الاخضر بما في ذلك مقدرته علي قتل البكتيريا. وفي هذه الدراسة تم استخدام الاشريكية القولونية والعنقودية الذهبية لمعرفة تأثير الكاتشينات عليها بدون اضافة الملح وباضافته لتبين الدراسة ان للشاي الأخضر خاصية مضاد البكتيريا وتزيد هذه الخاصية عند اضافة الملح حيث يتحول لمادة فتاكة بالبكتيريا مما يساعد في محاربة عدد من الامراض. واتضح ان حساسية الاشريكية القولونية لمستخلص الشاي الاخضر اكبر من حساسية العنقودية الذهبية التي لم تسجل اي تثبيط في النمو الا بعد اضافة الملح ومن المحتمل ان يكون لطريقة الاستخلاص دور في ذلك.....

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# **Chapter One**

# Introduction

# INTRODUCTION

Throughout the history of human civilization, man has selected three important non-alcoholic beverages from nature's resources, namely tea, coffee and cocoa. Among these, tea is the most widely consumed beverage. It is consumed by half of the world's population for its attractive aroma, taste and health benefits. It is a safe and easily affordable drink for all sections of society throughout the world and there is considerable evidence that consumption of tea is one of the most important ways to prevent a number of human ailments. It has evoked great interest in the medical community in the past few decades. Scientific research has validated the positive effects of tea on health, especially green tea, and shifted its reputation from being "the cup that cheers" to "the cup that heals".

## 1.1 Green Tea Plant:

Scientific names: *Camellia Sinesis*, Kuntze. Family: *Theaceae*

Common name: Green tea.

## 1.2 Description:

*Camellia sinensis* is native to East Asia, the Indian Subcontinent and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions.

*Camellia sinensis* is an evergreen shrub or small tree that is usually trimmed to below 2 m (6.6 ft) when cultivated for its leaves. It has a strong taproot. The

flowers are yellow-white, 2.5–4 cm (0.98–1.57 in) in diameter, with 7 to 8 petals.

The seeds of *Camellia sinensis* and *Camellia oleifera* can be pressed to yield tea oil, a sweetish seasoning and cooking oil that should not be confused with tea tree oil, an essential oil that is used for medical and cosmetic purposes, and originates from the leaves of a different plant.

*Camellia sinensis* plant, with cross-section of the flower (lower left) and seeds (lower right)

The leaves are 4–15 cm (1.6–5.9 in) long and 2–5 cm (0.79–1.97 in) broad. Fresh leaves contain about 4% caffeine, as well as related compounds including theobromine. The young, light green leaves are preferably harvested for tea production; they have short white hairs on the underside. Older leaves are deeper green. Different leaf ages produce differing tea qualities, since their chemical compositions are different. Usually, the tip (bud) and the first two to three leaves are harvested for processing. This hand picking is repeated every one to two weeks. [1]

## 1.3 Green Tea History:

Over the past fifty years, green tea has exploded into an extremely popular beverage choice for tea drinkers world wide. But despite its late entrance into the western world, green tea has been consumed for centuries in Asian countries like China, Japan and India. Along with most other types of tea, green tea began as a drink fit only for nobles and slowly became available for all levels of society. In recent years, green tea's health benefits have made it even more popular among tea drinkers, especially in the western hemisphere. [2]

## 1.4 Amazing legends about Green tea:

Because of its popularity in Asia and its recent date popularity in the west, many stories, myths as legends have circulated about green tea, especially in china. Two of them, very interesting tales, tell the story of who green tea firstly originated in china.

One such legend tells the story of a Chinese emperor called Shen Nung, who had a habit of boiling the water before drinking it. He first tasted green tea after a tea blossom fell into his cup of hot water by accident. He was so pleased with its taste and properties that he ordered that the small tea trees should be planted in the palace's gardens establishing a tradition of tea drinking at the Chinese royal court.

Another Chinese legend about green tea discovery was about a man Shien Non Shei on his name, who one day took his wife and children mountain climbing. During the climb, a leaf brought by wind, drifted onto his feet. He tasted the leaf and thought it had an amazing taste and also felt that this leaf could have healing properties and could help quench thirst, when brewed. Thus, according to this legend he was the first individual to actually drink tea. [3]

## 1.5 A brief history about green tea:

The use of green tea as a health aid goes all the way back in time to the year 2737BC. Chinese elite and bureaucrats were the ones who could afford green tea, because at the time, being a new discovery, tea was really expensive and not at all a common drink as we think of it nowadays. They also included green tea among their dining habits. We can say about green tea that it become number two drink in the world without us even realizing it. It was love at first sight.

After being discovered, the tea leaves undoubtedly became a custom. The Chinese learned and understood green teas health benefits. Elaborate tea ceremonies were born becoming a traditional celebration of nature, etiquette and health.

Green tea eventually found its way to Japan in between 729 and 800 AD. The Japanese emperor gave powdered green tea as gifts to Buddhist monks. The monks started to use green tea in elaborate ceremonies making tea an important drink for Japanese also. In a few years they also started to cultivate it and produced their own tea varieties competing with the Chinese tea market. [4]

## 1.6 Green Tea Goes West:

While green tea has been enjoyed in china for around 5000 years, its history in the west is relatively short in comparison. One of the most interesting aspects of green tea history is its slow move from Asia to Europe and America.

European traders where first introduce to tea in the sixteenth century during trips to East Asia. The new drink so pleased the sailors and their homelands that it become an important commodity. Even now, great Britain's national beverage is tea, though most British tea drinkers prefer black teas.

Along with settlers, tea was shipped over to the Americas, where it enjoyed great popularity among the early colonists. In fact, tea was so popular in America that great Britain imposed a Tea Tax in 1767 that infuriated the colonists and sparked the Boston tea party of 1773, where 45 tons of green tea (called "bullet" tea because of its shape during shipping) was dumped into the harbor. [5]

## 1.7 Green Tea Ingredients

Are extremely complex. It contains as many as 200 bioactive compounds. Over the past 20 years, scientists discovered that most of its health benefits can be explained by the big three:

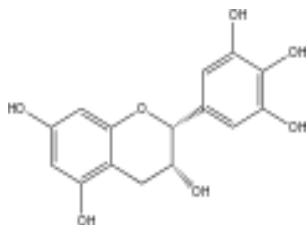
- Polyphenols.
- Theanine.
- Caffeine.

The largest and most important chemical compound is polyphenols. Because they contain flavonoids(an important class of antioxidants)

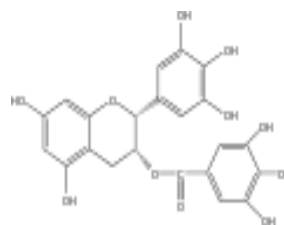
Catechin:

The most important nutrition in tea polyphenols are catechins. According to scientist Graham Harold dried tea extract can contain 30% to 40% catechins, the four main catechins are:

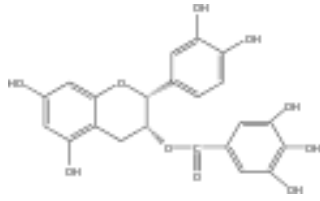
- ❖ Epicatechin(EC)
- ❖ Epicatechin-3-gallate(ECG)
- ❖ Epigallocatechin(EGC)
- ❖ Epigallocatechin-3-gallate(EGCG)



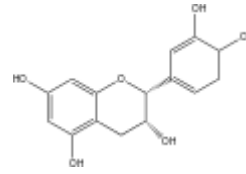
(-) -[Epigallocatechin](#)



(-) -[Epigallocatechin gallate](#)



(-) [-Epicatechin gallate](#)



(-) [-Epicatechin](#)

Figure 1.1 structure of catechins

## 1.7.1 EGCG:

The star of the show of course is EGCG found in the highest concentration in green tea, is the most active and best researched of all green tea ingredient.

But it simply, it explains most of green tea health benefits it has been found to be over 100 times more effective in neutralizing free radical than vitamin C and 25 times more powerful than vitamin E.

It also tops other antioxidants such as (BHA) butylated hydroxyanisole,(BHT) butylated hydroxytoluene and resveratrol. [6]

## 1.7.2 Other Polyphenols:

Dried leaves can contain 7% to 14% of other flavonoids compounds. About two thirds of these flavonoids are flavonols.Such as kaempferol,quercetin and myricetin. Also powerful antioxidants, they are known to be anti-histamine and anti-inflammatory.

Dry green tea contains 2% to 3% of theogallin which is unique only to tea. It also contains daysides such as chlorogenic acid and coumarylquinic acid. [7]

### 1.7.3 Theanine:

Dried tea extract contains 4% to 6% of theanine an amino acid found only in tea. It is what gives tea the charecteristic flavor. Catechins and caffeine taste bitter and astringent, but theanine tastes sweet and fresh. [8]

### 1.7.4 Caffeine:

IS a plant alkaloid found in coffee, tea and cocoa. It acts as natural pesticide, protecting plants against certain insects feeding on them.

Green tea contains alkaloids known as methylxanthines such as caffeine, theobromine and theophylline.

Graham found that fresh leaves contain on average 3% to 4% of caffeine and very small amounts of the other methylxanthines. [9]

### 1.7.5 Vitamins and minerals:

Green tea contains several B vitamins and C vitamin being less processed than black tea.

Other green tea ingredients include 6% to 8% of minerals such as potassium present in large amount, sodium, calcium, fluorine, aluminium, selenium, manganese and iron. [10]

### 1.7.6 Aromatic Oils:

There are 500 types of aromatic oils in green tea, it gives the charecteristic odour.



Green tea also contains organic acids such as gallic and quinic acid. [11]

### 1.7.7 Inorganic Elements:

Some specific inorganic elements present in tea are aluminium, fluorine and manganese. The level of aluminium and fluorine in tea leaves is higher than in other plants. It is presumed that the tea plant has a biochemical mechanism to neutralize the toxicity of aluminium. It has been observed that aluminium in tea leaf exists mainly in a chelated form, indicating that catechins prevent aluminium toxicity. These findings are important in the physiological significance of tea polyphenols. [12]

### 1.7.8 Carbohydrates and Lipids:

The carbohydrate content of green tea is about 40% and one third of it is cellulose. Starch is also present and affects the quality of green tea. Tea harvested in the morning has less starch and is of better quality in comparison to afternoon collections.

Tea leaves also contain about 4.0% oil. [13]

## 1.8 Benefits of Green Tea:

Green tea has been used as a medicine for thousands of years, originating in China but widely used throughout Asia this beverage has a multitude of uses from lowering blood pressure to preventing cancer. The reason that green tea has more health benefits attached to it than black tea is (apparently) due to the processing. Black tea is processed in a way that allows for fermentation whereas green tea's processing avoids the fermentation process. As a result, green tea retains maximum amount of antioxidants and poly-phenols the substances that give green tea its many benefits.

Here's a list of some of its amazing benefits — benefits that you may not have been aware of. Some of these benefits are still being debated, so please do your own research if you want to use green tea for medicinal purposes.

- **Weight Loss.** Green tea increases the metabolism. The polyphenol found in green tea works to intensify levels of fat oxidation and the rate at which your body turns food into calories.
- **Diabetes.** Green tea apparently helps regulate glucose levels slowing the rise of blood sugar after eating. This can prevent high insulin spikes and resulting fat storage.
- **Heart Disease.** Scientists think green tea works on the lining of blood vessels, helping keep them stay relaxed and better able to withstand changes in blood pressure. It may also protect against the formation of clots, which are the primary cause of heart attacks.
- **Esophageal Cancer.** It can reduce the risk of esophageal cancer, but it is also widely thought to kill cancer cells in general without damaging the healthy tissue around them.
- **Cholesterol.** Green tea reduces bad cholesterol in the blood and improves the ratio of good cholesterol to bad cholesterol.
- **Alzheimer's and Parkinson's.** It is said to delay the deterioration caused by Alzheimer's and Parkinson's. Studies carried out on mice showed that green tea protected brain cells from dying and restored damaged brain cells.
- **Tooth Decay.** Studies suggests that the chemical antioxidant “catechin” in tea can destroy bacteria and viruses that cause throat infections, dental caries and other dental conditions
- **Blood Pressure.** Regular consumption of green tea is thought to reduce the risk of high blood pressure.

- **Depression.** Theanine is an amino acid naturally found in tea leaves. It is this substance that is thought to provide a relaxing and tranquilizing effect and be a great benefit to tea drinkers.
- **Anti-viral and Anti-bacterial.** Tea catechins are strong antibacterial and antiviral agents which make them effective for treating everything from influenza to cancer. In some studies green tea has been shown to inhibit the spread of many diseases.
- **Skincare.** Green tea can apparently also help with wrinkles and the signs of aging; this is because of their antioxidant and anti-inflammatory activities. Both animal and human studies have demonstrated that green tea applied topically can reduce sun damage. [14]

# Literature review

First cultivated in China nearly 5,000 years ago, tea is consumed in greater quantity worldwide than any other beverage except water. The beverage is made from the leaves of the plant *Camellia sinensis*, (family Theaceae), which is native to India and perhaps parts of China and Japan. Black, green and oolong teas are all made from this plant but differ in their methods of preparation.

All tea leaves are withered, rolled and heated, but black teas go through an oxidative process known as fermentation before the final heating process. Oolong teas are partially fermented. The secondary metabolites found in the tea plant and the unique combinations of these secondary metabolites are responsible for the popularity of this crop as source of consumed soft beverage. Components of the tea such as epigallocatechin gallate, epicatechin gallate, epicatechin act as effective scavengers of free radicals. [15]

It has been shown that natural antioxidants in tea possess stronger antioxidative activity than synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and dl- $\alpha$ -tocopherol interestingly, tea polyphenols are much less toxic than that of butylated hydroxyanisole, butylated hydroxytoluene and dl- $\alpha$ -tocopherol . India is a major tea (black tea) producer in world followed by Japan (green tea) and China (different sorts of tea). Green tea is generally safe, non toxic and having no side effects after use. Catechins from tea extracts have been observed to be cytotoxic to microbial pathogens and therefore may be useful as antibacterial agents.

There is growing evidence that indicates that the catechin components of green tea are responsible for the observed antibacterial activity, and that Epigallocatechin, Epigallocatechin gallate and Epicatechin gallate constitute the

most important antibacterial agents. Black tea which is a major source of phenolics, including theaflavins and thearubigin has also been shown to have antibacterial properties both *in vivo* and *in vitro*. [16]

The development of antibiotic resistance in bacteria is a major issue in the prevention of infectious diseases. Currently the spread of multi-drug resistant bacteria is not only through nosocomial infections, but also occur in the community. Predominant multi-resistant bacteria that causes the infection such as *P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) have been found in several hospital. As the bacteria that cause the infection was resistant to first-line antibiotics, treatment options are usually replaced with a second or third choice of antibiotics, which are generally much more expensive.

Therefore, alternative antimicrobial agents are needed to be developed and employed to control multi-drug resistant bacteria. To face this challenge, there has been growing interests to find antimicrobial compounds from medicinal plant extracts as an alternative approach to discover new antimicrobial compounds. The antimicrobial activities of some herbal medicines against different pathogens have been reported from different countries. *Camellia sinensis* (*C. sinensis*), has been reported to have antimicrobial activities against various pathogenic bacteria. Urinary tract infections play a significant role in transmission of drug resistance, as these infections present in asymptomatic form and are, caused by opportunistic pathogens of intestinal tract. The present study was designed to check antibacterial activity of tea extracts against bacteria isolated from urine samples of patients suffering with UTI. The isolates were also checked for expression of virulence features. This will helps us to see the antibacterial activity of tea against pathogenic bacteria and to design chemotherapy against the disease caused by them. [17]

# Objectives:

- 1- Isolation of catechin compounds from green tea.
- 2- Determine the sensitivity and antibacterial activity of green tea extract against *Escherichia coli*, *Staphylococcus aureus*.

## **Chapter two**

# Material and methods

## 2. Material and methods

### 2.1 Material:

Cofftea green tea bag, distill water, chloroform, ethyl acetate, table salt, *E.coli*, *Staphlococcus aureus*.

### 2.2 Apparatus:

Sensetive balance, separatory funnel, conical flask, beaker, filter paper, glass rod, funnel, mueller hinton agar plates, filter disc (6mm), incubator, membrane filter.

### 2.3 Methods:

#### 2.3.1 Extraction of components from green tea

Initially, 3 g of ground leaves of green tea was extracted with 60 ml of pure water at temperature of 80 C, for 40 min. The extraction was filtered by filter paper. The filtered samples were initially partitioned with water/chloroform (1:1 vol. %). Then the water phase was collected and the impurities associated with the chloroform phase were discarded. As a second partition, water/ethyl acetate (1:1 vol. %) was used. Catechin compounds moved into the ethyl acetate layer and were collected in weighed beaker. [18]



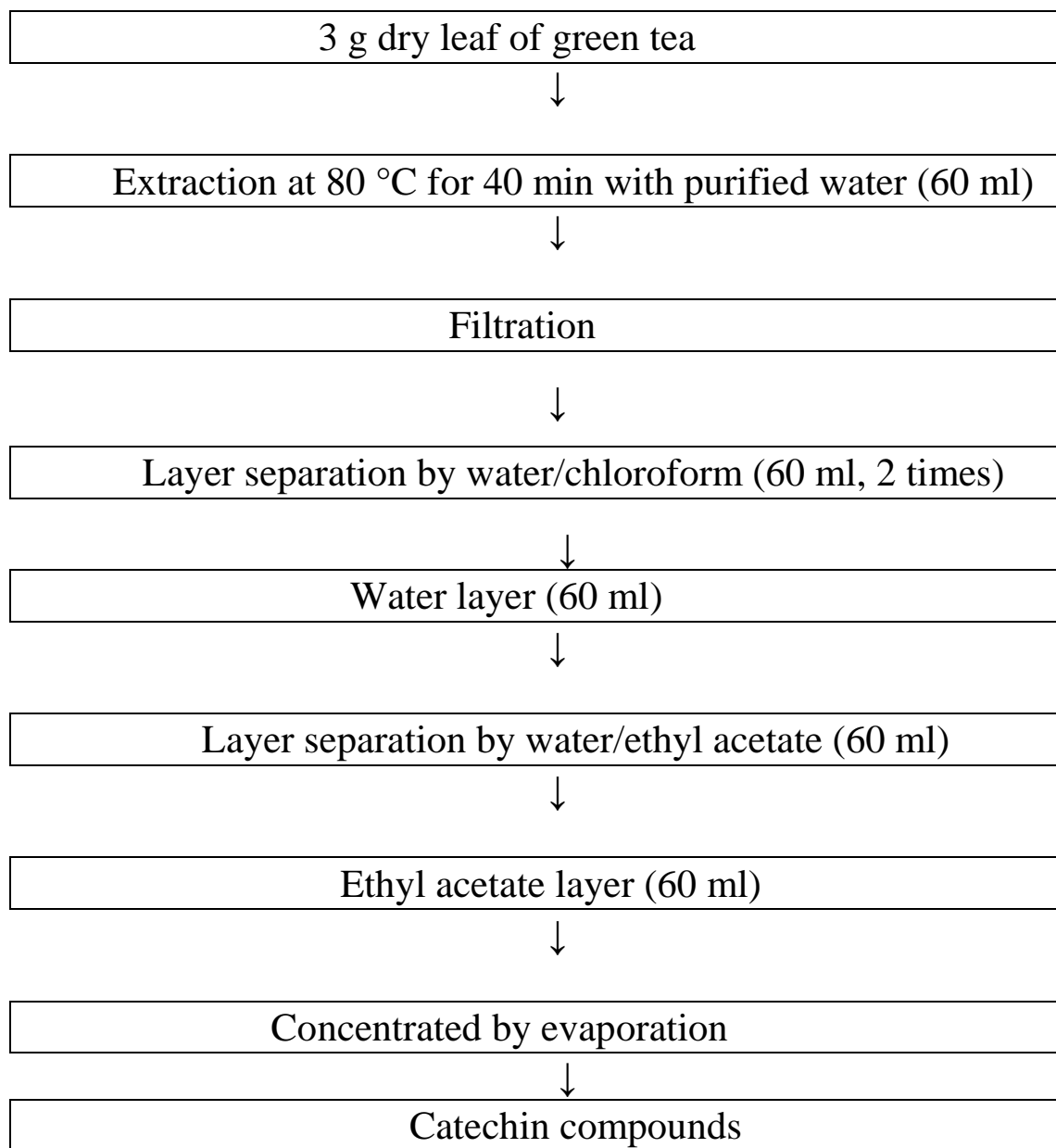


Figure 2.1 Scheme of the recovery of catechin compounds by solvent extraction from green tea.

## 2.3.2 Preparations of plant extracts:

Certain concentration of the plant extract was prepared by dissolving it with sterile distilled water, and another extract was prepared by adding table salt, and filtrated through a membrane filter. [19]

## 2.3.3 Antibacterial activity test:

Antibacterial activity was determined using the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines. The bacteria used in this study were *S. aureus*, *E.coli*, the clinical isolations which were obtained from the Laboratory of Clinical Microbiology, Laboratory Administration.

The dried plant extracts were dissolved in sterile distilled water, and another extract was prepared by adding table salt, and sterilized by filtration through a membrane filter.

Pre-warmed Mueller-Hinton agar plates were seeded with tested bacteria. An aliquot of plant extract solutions were pipetted onto sterile paper discs (6 mm diameter) and placed onto the surface of inoculated agar plates. Plates were incubated at 37 °C for 24 h. Antibacterial activity was expressed as the diameter of the inhibition zone (mm) produced by the extracts around the disc. [20]

# **Chapter Three**

## Results and Discussion

## 3. Results and discussion

### 3.1 Percentage yield of catechin compounds:

The present study showed that the total content of catechin compounds in coffee green tea is 3.90%. In this study, the ground leaves of green tea were extracted under the experimental conditions at temperature of 80 C, for 40 min using water as solvent under continuous stirring. Besides the target compounds, many other compounds (impurities) were also extracted. Using the water–chloroform partition, caffeine and related impurities extracted into chloroform those were removed. The more partitioning that was done, the more caffeine appeared be removed. After the water–chloroform partition, another partition step using water–ethyl acetate as the solvents was applied for further purification. Catechin compounds such as EC, EGC, EGCG, and ECG migrated onto the ethyl acetate layer and were collected.

Similar results were obtained with water and ethanol under lower temperatures.

But to get pure catechin compounds it is better to use water, because water is friendly to the environment and can be applied at higher temperatures, it was chosen as the final extraction solvent.

### 3.2 Antibacterial activity test results:

A large variety of research has been performed assessing whether or not green tea has antimicrobial activities. A summary of examples of this research is included in **Table1**. The research results shown do suggest that green tea may be effective against many organisms. The research is pretty well-balanced between gram positive and gram negative organisms.

A new study done in Northwestern university shows that when we add table salt to green tea extract it becomes more effective against bacteria.

The present study showed that the *E.coli* is sensitive to GTE (the inhibition zone is 18mm). And exhibits more sensitivity when we add table salt (20mm).

*E.coli* is more sensitive to GTE in contrast with *S.aureus* which is not sensitive to GTE only (No zone of inhibition). *S.aureus* exhibits sensitivity to GTE + table salt (the inhibition zone is 12mm). And this indicates that adding of table salt increases the antimicrobial activities of green tea.

**Table (1):**

**Antimicrobial activity of green tea extracts on *E.coli* and *S.aureus***

Isolates tested	Zone of inhibition in mm	
	GTE	GTE+Table salt
<i>E.coli</i>	<b>18</b>	<b>20</b>
<i>S.aureus</i>	<b>No zone</b>	<b>12</b>

**GTE= green tea extract.**

**E.coli= Escherichia coli.**

**S.aureus= Staphylococcus aureus.**

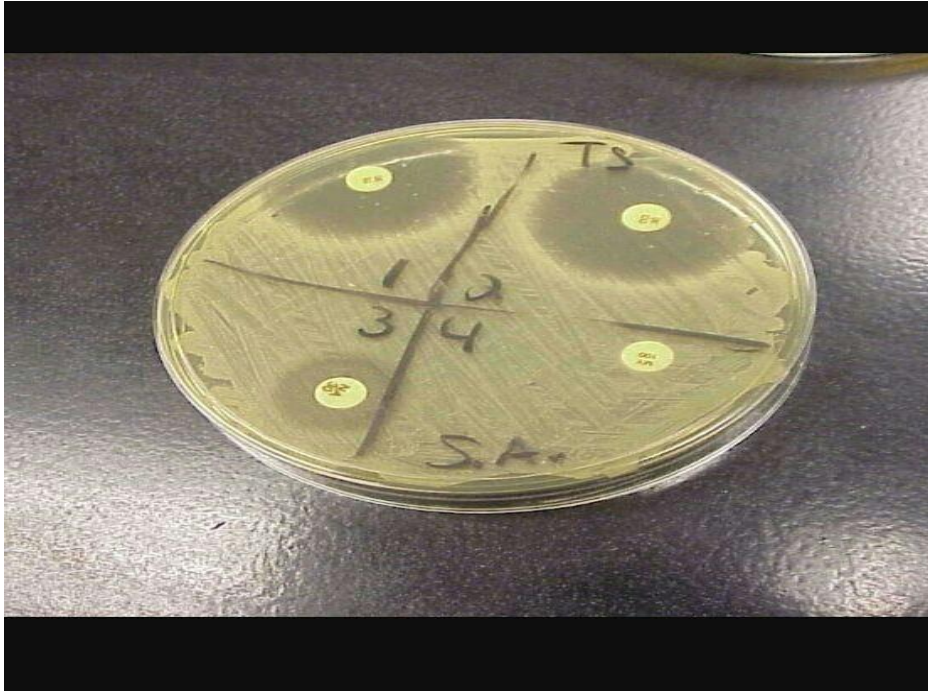


Figure 3.1 describes the killing activities of green tea extract against *E.coli* and *S.aureus*. The label 1&2 for *e.coli*. 3& 4 for *s.aureus*.

# Conclusion

According to the results of this study it was concluded that:

1. The optimal procedure to obtain catechin compounds from the leaves of green tea by optimizing operating factors, such as, the extraction solvent, extraction time and operating temperature were established. The extract was partitioned between water–chloroform, to remove caffeine impurities. Finally, the resulting extract was partitioned with water–ethyl acetate to purify the catechin compounds of EGC, EC, EGCG and ECG. These results could be extended to preparative HPLC to obtain EGCG on a commercial scale.
2. This experiment has shown that green tea in general have antimicrobial properties. The antimicrobial properties increase with adding table salt.
3. The lack of antimicrobial properties for the tea sample against *S.aureus* may have been due to preparation method.

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