Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [5]April 2023 : 185-194

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Anti-Inflammatory Effect of *Camellia sinensis* using *in-vitro* study

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Abstract
The goal of the current study was to assess the preliminary phytochemical and anti-inflammatory effects of Camellia sinensis using phytochemical parameters of the plant's leaf, including loss of drying, ash value, acid soluble ash, water and alcohol soluble extractive values, qualitative and quantitative test, and in-vitro protein denaturation methods. A preliminary phytochemical screening test was carried out using petroleum ether, water, methanol, and chloroform. According to the phytochemical results data, methanol extract contained the maximum concentration of phyto-compounds, and all other pharmacognostic parameters were within the Indian Pharmacopoeia's acceptable range. The in-vitro % inhibition of protein denaturation method was used to study and assess the anti-inflammatory properties of Camellia sinensis extracts. In order to evaluate the anti-inflammatory property, the test extracts were incubated with egg albumin at various concentrations under strictly controlled experimental circumstances. The reference medication utilised was acetyl salicylic acid. The current findings showed that extracts of methanol exhibited highest inhibited protein (albumin) denaturation in a concentration-dependent manner. In the normal group, standard group, methanol extract (100 g/ml), methanol extract (250 g/ml), and methanol extract (500 g/ml), the percentage inhibition of proteinase activity of Camellia sinensis and was found to be 66.3%, 50.0%, 35.15%, 52.35%, and 58.15%, respectively. All things showed that methanolic extract of Camellia sinensis having strong anti-inflammatory bioactive components and that can aid to lessen inflammation and fend off chronic illnesses. To get the most anti-inflammatory benefits from Camellia sinensis, however, additional research is required to discover the ideal dosage and time frame for consumption. The findings of this research offer insightful information about the phytochemical makeup and anti-inflammatory activities of Camellia sinensis, which may be helpful in the creation of innovative therapeutic agents for the treatment of chronic inflammatory illnesses.
Keywords: *Camellia sinensis, anti-inflammatory, herbal plants, Thin layer chromatography.*

Received 21.02.2023 Revised 15.03.2023 Accepted 21.04.2023

INTRODUCTION

Medicinal plants have been used for thousands of years to treat various health conditions and diseases. They contain active compounds that have therapeutic properties and can be used to promote healing and alleviate symptoms. Different parts of the plant may contain different active compounds, such as the leaves, flowers, roots, or bark. Medicinal plants can be consumed in various forms, including teas, tinctures, capsules, and extracts.[1,2] In recent years, there has been a growing interest in natural remedies and alternative medicine, leading to increased research on the medicinal properties of plants. Many modern medicines are derived from medicinal plants, and scientists continue to study the potential of these plants for future medical applications. However, it is important to note that not all medicinal plants are safe and effective for everyone. Some may interact with medications or cause side effects, and their use should always be discussed with a healthcare provider. Overall, medicinal plants offer a natural and potentially effective alternative to conventional medicine, and their use has been an important part of traditional medicine for centuries.[3,4]

Camellia sinensis is a species of evergreen shrub or small tree that is native to East Asia, including China, Japan, and Korea. It is best known as the plant from which tea is made, and is commonly referred to as the

tea plant or tea tree. *Camellia sinensis* has dark green, glossy leaves and produces fragrant, white flowers in the fall. The leaves are harvested and processed to create different types of tea, including green tea, black tea, white tea, and oolong tea. The specific type of tea produced depends on how the leaves are harvested and processed. [4, 5] In addition to being a popular beverage, *Camellia sinensis* has also been used for medicinal purposes in traditional Chinese and Ayurvedic medicine. It contains several compounds that are believed to have health benefits, including caffeine, theanine, and antioxidants [6].

Inflammation is a biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants. The main purpose of inflammation is to protect the body by eliminating the initial cause of cell injury, clearing out necrotic cells and tissues damaged from the original insult, and initiating the tissue repair process. [7] The inflammatory response involves the activation of immune cells, such as white blood cells and cytokines, which release chemicals that increase blood flow to the affected area and cause swelling, redness, heat, and pain. This response is part of the body's natural defense mechanism against infection and injury, but chronic inflammation can lead to tissue damage and disease. [8] Inflammation can occur in response to various factors such as infections, injuries, allergies, autoimmune disorders, and exposure to toxins. Common symptoms of inflammation include redness, swelling, warmth, pain, and loss of function in the affected area. Treatment of inflammation typically involves addressing the underlying cause and may include medications such as anti-inflammatory drugs, corticosteroids, or immunosuppressants.[9] Inflammation is a natural response of the immune system to protect the body against harmful stimuli, such as pathogens, injuries, or toxins. There are two main types of inflammation: acute inflammation and chronic inflammation [10-16].

MATERIAL AND METHODS

Plant material was fresh Plant leaf of *Camellia sinensis*. It needs to be crushed, using a pestle and mortar, to provide a greater surface area. The plant material should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of *Camellia sinensis* leaves in a 25- x 80-mm thimble).

Soxhlet Extraction

All equipment was provided for me to assemble. Begin by building a rig using stands and clamps to support the extraction apparatus. Following this, the solvent was added to a round bottom flask, which is attached to a Soxhlet extractor and condense on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 16 hours [17].

Determination of Physico-Chemical Parameters

Ash values

Total ash and acid-insoluble ash contents are important indices to illustrate the quality as well as purity of herbal medicine. Total ash includes ``physiological ash'', which is derived from the plant tissue itself, and ``non-physiological ash'', which is often from environmental contaminations such as sand and soil.

Total ash

1 g of powdered drug was accurately weighed and taken in tarred silica crucible which was previously ignited and weighed. The powdered drug was scattered into a fine layer at the bottom of the crucible and incinerated gradually by increasing the heat not exceeding dull red heat, cooled and weighed.

Weight accurately a cantered crucible after ignition and cooling = a gram.

Weight of crucible + 03 gram of powder = b gram.

Weight of powder (b-a) = X gram.

After incineration, weight of crucible + powder remaining = c gram.

Weight of ash = c-a gram.

% ash content = c-a/X × 100

Acid Insoluble Ash Value

Acid insoluble ash is used to measure the amount of minerals, such as silica, that are present in a sample. The ash obtained as directed under total ash was boiled with 25 ml of dilute HCl for 5 minutes.

Water Soluble Ash Value

The water soluble ash value is a measure of the amount of inorganic material that is present in the plant ash. This value is important because it can help to determine the extractive values of the plant ash. The water soluble ash value is determined by taking a sample of the ash and adding water to it.

Water soluble ash (%w/w) = (weight of ash – weight of insoluble ash/ weight of sample) ×100

Alcohol Soluble Ash value

The ASA value is important because it helps to determine how much of the plant's mineral content is extractable. This information is valuable for both farmers and processors who need to know how much of the plant's nutrients can be extracted and used.

Alcohol soluble extractive = value (%w/w)

Loss on Drying

Glass stoppered shallow bottle was weighed that had been dried in the same conditions to be employed in the determination. Nearly 1 gm of the sample was transferred to the bottle, closed and accurately weighed. The samples were distributed as evenly as practicably gently side wise shaking to a depth not exceeding 10 mm.

LOD Calculation:

$$\text{Loss on drying (\% w/w)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

Where,

W1: Weight of empty LOD bottle in g

W2: Weight of LOD bottle with Sample before drying in g

W3: Weight of LOD bottle with sample after drying in g.

Phytochemical Screening

Detection of Carbohydrate

500 mg of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch's Test

To 1 ml of filtrate, 2 drops of Molisch's reagent was added in a test tube and 2 ml of concentrated sulphuric acid added carefully along the side of the test tube. Furfural compounds are easily formed from all carbohydrates, when treated with conc. H_2SO_4or HC1 and it can be given purple-violet coloured compounds in the presence of α-naphthol.

Fehling's Test

To 1 ml of filtrate, 4 ml of Fehling's solution was added in a test tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

Fehling's solution

34.66 gm of copper sulphate was dissolved in distilled water and volume was made up to 500 ml.

b) 173 gm of potassium sodium tartarate and 50 gm of sodium hydroxide were dissolved in distilled water and made up to 500 ml.

c) (a) and (b) solutions were mixed in equal volume to give Fehling's solution.

Benedict' Test

To a clean and dry test tube containing about 3 ml of sample solution, 2-3 ml of Benedict's qualitative reagent is added and then boiled for 5 minutes. The solution turns green, yellow and finally Red, depending on the amount of reducing sugar present in the sample.

Barfoed's Test

About 1 ml of sample solution is added to about 3 ml of barfoed's reagent is a test tube and then boiled for 1-2 minutes add finally cooled. Red precipitate of Cu_2O is formed at the bottom of the test tube. Presence of reducing sugar in the supplied sample (Monosaccharides are more sensitive than reducing disaccharides).

Trommer's Test

About 1 ml of 2.5% $CuSO_4$ solution and 2 ml of 5% NaOH solution are added to 3-5 ml of carbohydrate solution. The mixture is boiled in a water bath for 3-5 minutes. A blue precipitate of Cupric hydroxide is formed which dissolves to give a blue solution. A yellow or red precipitate of Cu_2O is formed in presence of reducing sugar.

Moore's Test

To about 2 ml of sample solution, an equal volume of 5% NaOH solution is added and then boiled for 2-5 minutes in a water bath. The solution turns yellow and then reddish-brown due to the formation of a condensation product of the sugar. The sample contains reducing sugar, particularly glucose.

Detection of Glycosides

0.5 gm of extract was hydrolyzed with 20 ml of dilute hydrochloric acid (0.1N) and filtered. The filtrate was used to test the presence glycosides.

Modified Borntrager's Test

To 01 ml of filtrate, 02 ml of 1% ferric chloride solution was added in a test tube and heated for 10 minutes in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. Formation of rose

pink or cherry colour in the ammonia layer indicates the presence of glycoside.

Borntrager's test

1gm of drug sample + 5-10 ml of dilute HCl + 10 min. boil on waterbath and filter + extract of filterate with CCl4 or benzene + equal amount of ammonia solution to filterate + shake → appearance of pink to red colour → indicate presence of anthraquinone moiety.

Cuprocyanate test

Saturate the filter paper in freshly prepared solution of guaic resin + dissolved in ethanol → dry. → make contact of that filter paper with dilute solution of CuSO4 →place it with contact of drug sample → Generation of HCN gas with the appearance of stain → the presence of cynogenetic glycoside.

Feeriferrocyanide test

1 gm of drug sample + 5 ml of alcoholic KOH → transfer it to aqueous solution of FeSO4 and FeCl3 → keep it on room temperature for10 minutes. → transfer whole solution to 20 % HCl →appearance of Prussian blue colour → the presence of cynogenetic glycosides.

Shinoda test

Alcoholic extract of 1 gm of drug + magnesium turning+ dilute HCl → appearance of red colour → the presence of flavonoids.

• alcoholic extract of 1 gm of drug sample + zinc turning + dil. HCl →appearance of deep red colour → turns to magenta colour → the presence of dihydro flavonoids(other type of flavonoid glycoside).

Ammonia test

To the alcoholic solution of 1 gm of drug sample, when filter paper dipped and after that exposed to ammonia vapor, appearance of yellow spot on the filter paper indicates the presence of flavonoid.

Killer Killiani Test

Small portion from the respective extracts was shaken with 1 ml glacial acetic acid containing a trace of ferric chloride. 1 ml of conc. sulphuric acid (H2SO4) was added carefully by the sides of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquids indicate the presence of glycosides.

Legal's test

Small portion of extract was treated with dilute hydrochloric acid for few hours on a water bath and to the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside were added and sodium hydroxide solution to make it alkaline. Presence of pink to red clour shows the presence of glycosides.

Detection of Alkaloids

0.5 gm of extract was dissolved in 10 ml of dilute hydrochloric acid (0.1 N) and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's Test

Filtrates were treated with Mayer's reagent; formation of yellow cream coloured precipitate indicates the presence of alkaloids. Mayer's reagent is used in this test to detect the presence of alkaloids. It is prepared by dissolving mercuric chloride and potassium iodide in water. A cream-colored complex is created that appears in the form of precipitates. Precipitate formation confirms the presence of alkaloid in the sample.

Mayer's reagent

Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution) to give a cream coloured precipitate.

Dissolve 1.36 gm of mercuric chloride in 60 ml of distilled water. Dissolve 5 gm of potassium iodide in 20 ml distilled water. Mix (a) and (b) and adjust the volume to 100 ml with distilled water.

Dragendorff's Test

Filtrates were treated with Dragendroff's reagent; formation of red coloured precipitate indicates the presence of alkaloids. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's test reagent) was added and shaken. An orange red precipitate formed indicates the presence of alkaloids in dragendorff's test.

Dragendorff's reagent

Dragendorff's reagent is a color reagent to detect alkaloids in a test sample or as a stain for chromatography plates. Alkaloids, if present in the solution of sample, will react with Dragendorff's reagent and produce an orange or orange-red precipitate.

Hager's test

Drug solution + few drops of Hagers reagent (Saturated aq. Solution of Picric acid), formation of crystalline yellow precipitate. Hager's reagent contains a saturated picric acid solution. The reaction gives yellow precipitate which confirms the presence of alkaloids.

Hager's reagent

Hager's reagent (saturated Picric acid solution) gives yellow color precipitate. ρ-dimethyl amino benzaldehyde uses for ergot alkaloids and pyrrolizidine alkaloids.

Saturated solution of picric acid in distilled water.

Wagner's test

Wagner's test iodine reacts with I- ion from potassium iodide producing $I3^-$ ion (brownish solution). 1mL of potassium mercuric iodide solution (Wagner's test reagent) was added and shaken. Emergence of whitish or cream precipitate implies the presence of alkaloids.

Wagner's reagent

An aqueous solution of iodine and potassium iodide; used for microchemical analysis of alkaloids Also kn own as Wagner's solution.

Detection of Phytosterols and Triterpenoids

0.5 gm of extract was treated with 10 ml chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpinoids.

Libermann-Buchard test

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

Libermann -Buchard reagent

Lieberman-Burchard is a reagent used in a colourimetric test to detect cholesterol, which gives a deep green colour. This colour begins as a purplish, pink colour and progresses through to a light green then very dark green colour. The colour is due to the hydroxyl group (-OH) of cholesterol reacting with the reagents and increasing the conjugation of the un-saturation in the adjacent fused ring. Since this test uses acetic anhydride and sulfuric acid as reagents, caution must be exercised so as not to receive severe burns. Dissolve one or two crystals of cholesterol in dry chloroform in a dry test tube. Add several drops of acetic anhydride and then 2 drops of concentrated $H2SO4$ and mix carefully. After the reaction is finished, the concentration of cholesterol can be measured using spectrophotometry.

Salkowaski Test

Salkowski's test, also known simply as Salkowski test, is a qualitative chemical test that is used in chemistry and biochemistry for detecting a presence of cholesterol and other sterols. This biochemical method got its name after German biochemist Ernst Leopold Salkowski, who is known for development of multiple new chemical tests that are used for detection of different kinds of molecules (besides cholesterol and other sterols also for creatinine, carbon monoxide, glucose and indoles). A solution that has tested positive on the Salkowski's test becomes red and gets yellow glow

To the test extract solution added few drops of conc. $H2SO4$ shaken and allowed to stand, lower layer turns reddish brown or golden yellow indicating the presence of triterpenes. A solution that has tested positive on this qualitative chemical test exhibits two distinct layers in a test tube; the upper layer (chloroform) gets blueish red to violet colour, while the layer of sulfuric acid becomes yellow to green, with greenish glow being visible. If a sample does not contain any cholesterol or other sterols the tested solution remains unchanged and retains its original colour.

Salkowaski reagent

The color developed by positive reaction indicates the presence of various indole compounds as a product of tryptophan metabolism.

Detection of Protein and Amino Acid

100 mg of each extract was taken in 10 ml of water and filtered. The filtrate was used to test the presence of protein and amino acids.

Ninhydrin Test

The amino acid proline, which contains a secondary amino group, reacts with ninhydrin. Blue-purple and yellow reaction products positively identify free amino groups on amino acids and proteins.

Biuret Test

A biuret test is a chemical assay that helps check for the presence of protein in a given sample. To confirm the presence of protein, it will rely on the changes in color.

Biuret reagent

Biuret test uses a reagent consists of potassium hydroxide and copper sulfate. Under normal condition, the color of the biuret reagent is blue. However, it changes its color to violet if peptide bonds are present.

Xanthoproteic Test

Some amino acids contain aromatic groups that are derivatives of benzene. These aromatic groups can undergo reactions that are characteristic of benzene and benzene derivatives. One such reaction is the

nitration of a benzene ring with nitric acid. The amino acids tyrosine and tryptophan contain activated benzene rings and readily undergo nitration. The amino acid phenylalanine also contains a benzene ring, but the ring is not activated and, therefore, does not readily undergo nitration. This nitration reaction, when used to identify the presence of an activated benzene ring, is commonly known as the xanthoproteic test, because the product is yellow.

Millon's Test

Millon's test is a test specific for tyrosine, the only amino acid containing a phenol group, a hydroxyl group attached to a benzene ring. In Millon's test, the phenol group of tyrosine is first nitrated by nitric acid in the test solution.

Millon's reagent

Dissolve 1 gm mercury in 9 ml of fuming nitric acid. Keeping the mixture well cooled during the reaction.

Detection of Flavonoids

Alkaline Reagent test

To 100 mg of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that becomes colorless on addition of few drops of dilute acid (HCl) indicates the presence of Flavonoids.

Detection of Saponin

Foam Test

Extracts were diluted with distilled water to 20 ml and Shaken in a graduated cylinder for 15 minutes. Formation of one cm layer of foam indicates the presence of Saponin distilled water.

Haemolysis test

Add 0.2 ml of solution of saponin (prepared in 1% normal saline) to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2ml of normal saline.

Thin-layer chromatography

Thin-layer chromatography is performed on a sheet of an inert substrate such as glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. The extracts were converted to crude extracts by distillation process with appropriate solvents. The movements of the active compounds were expressed by the retention factor (Rf). The Rf values were obtained from the phytochemicals provide the information about the polarity and separation of these phytochemical in the TLC separation process. Different Rf values of the phytocompounds also give the idea about their polarity by the use of the various solvent systems. The TLC plates were slightly dried and spots were detected with the help of UV light at 254 nm (lower wave length) and 366 nm (higher wave length).

Rf = Distance travelled by solute / Distance travelled by solvent.

Preliminary in-vitro anti-inflammatory assays

Protein denaturation assay

This assay was performed according to the protocol described earlier with some modification (Nguemnang et al., 2019). Reaction mixture was prepared by mixing varying concentrations of different extracts viz 100, 250 and 500µg/ml to 5% bovine albumin albumin in separate test tubes. Standard drug, Acetyl salicylic acid with standard drug was taken as positive control. Experiment was repeated three times and the percentage inhibition of protein denaturation exhibited by each extract was calculated as under:

$$\textbf{Percentage inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION

Organoleptic Properties of *Camellia sinensis*

Organoleptic is defined as being perceivable by the senses, such as smell, appearance, taste and touch. Organoleptic Properties of *Camellia sinensis* was determined and found to be Light Brown colour, Irregular shape, Distinctive Smell odour, Medium texture, Characteristic taste, respectively. The results are shown in Table 1.

Table 1: The organoleptic properties of *Camellia sinensis*

S.N.	Properties	Outcomes
1	Colour	Light Brown To Brown Powder
2	Shape	Irregular
3	Odour	Distinctive Smell
4	Texture	Medium
5	Taste	Characteristic

Figure 1: *Camellia sinensis* powder

Extraction of Selected *Camellia sinensis*

Extraction of Selected *Camellia sinensis* was prepared by using leaves (10.66 kg), different solvent water, pet ether, chloroform and methanol. The yield of extract was found to be 8.3%, 3.1%, 8.3% and 7.6 % respectively. The results of yield of extract indicated that highest bioactive components present in water, chloroform and methanol extract. These extract may have potential therapeutic activities.

Figure 2: Chloroform extract

Figure 3: Water extracts

Figure 4: Methanol extract

Table 2: Extractive values obtained from *Camellia sinensis*

S.N.	Solvent	Color of extract	% yield
1	Water	Dark Bluish	8.3%
2	Pet ether	Blackish	3.1%
3	Chloroform	Dark Bluish	8.3%
4	Methanol	Dark Bluish	7.6 %

Physicochemical Standardization of *Camellia sinensis*

The physicochemical parameters total Ash value, acid insoluble ash value, water-soluble ash value, moisture content, and foreign organic matter which was determined respectively. The results are shown in Table 3, 4, 5, 6, Fig. 6, 7, and 8 respectively. Total ash value, water soluble ash, acid insoluble ash, heavy metals, water soluble extractive, alcohol soluble extractive, and acidity were among the physicochemical parameters of raw materials assessed and found according to WHO criteria (pH).

Table 3: Standardization parameters of *Camellia sinensis* methanol extract

S.N.	Parameters	% value(w/w) *Camellia sinensis*
1	Total ash	7.52 %
2	Acid insoluble ash	2.30 %
3	Water soluble ash	6.20 %
4	Loss on drying	5.50 %
5	Foreign matter	0.5%

Table 4: Standardization parameters of *Camellia sinensis* water extract

S.N.	Parameters	% value(w/w) *Camellia sinensis*
1	Total ash	5.01 %
2	Acid insoluble ash	3.18 %
3	Water soluble ash	16.2 %
4	Loss on drying	4.12 %
5	Foreign matter	0.1 %

Table 5: Standardization parameters of *Camellia sinensis* chloroform extract

S.N.	Parameters	% value(w/w) *Camellia sinensis*
1	Total ash	6.01 %
2	Acid insoluble ash	2.25 %
3	Water soluble ash	17.3 %
4	Loss on drying	3.12 %
5	Foreign matter	1.15 %

Table 6: Standardization parameters of *Camellia sinensis* pet ether extract

S.N.	Parameters	% value(w/w) *Camellia sinensis*
1	Total ash	5.2 %
2	Acid insoluble ash	1.3 %
3	Water soluble ash	13.23 %
4	Loss on drying	2.13 %
5	Foreign matter	0.63 %

Table 7: Phytochemical constituents of *Camellia sinensis* extract

S.N.	Constituents	Water	Ethanol	Chloroform	Methanol
1	Alkaloids	+	+	-	+
2	Flavonoids	+	+	-	+
3	Phenols	+	+	-	+
4	Tannins	+	+	-	+
5	Saponins	+	+		+
6	Steroids & terpenoids	+	-	+	+
7	Glycosides	-	-	-	-
8	Carbohydrates	+	+	-	+
9	Anthraquinones	+	+	-	+

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

Phytochemical constituents of *Camellia sinensis* extract was determined and found to be highest bioactive constituents present in water, methanol and ethanol respectively, due to highest polar nature of solvent. It indicated that these solvent having highest potential for the pharmacological action.

Thin layer chromatography

The leave extracts of *Camellia varieties* indicated the presence of active biological compounds such as flavonoids, proteins, phenols, alkaloids and glycosides. In TLC analysis, different absorbing bands were observed under UV light (254 nm and 336 nm). Mostly, spots of yellow, green, brown, dark green colours were observed on TLC plates which indicated the presence of flavonoids, phenols, glycosides and alkaloids, respectively. The results are shown in Table 8 and Fig. 10.

Table 8: Rf value of ethanol extract of *Camellia sinensis*

S.N.	Sample	Rf Value	Colour	Resolution
1	A	0.06	Yellow	Good
2	B	0.72	Green	Excellent
3	C	0.12	Brown	Best
4	D	0.78	Dark green	Excellent

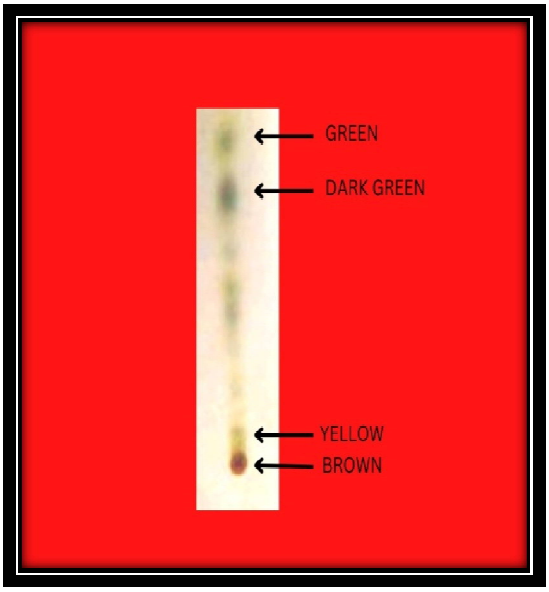

Figure 10: TLC of *Camellia sinensis* extracts

***In-vitro* anti-inflammatory effects of *Camellia sinensis* extracts**

Effect of *Camellia sinensis* extracts on protein denaturation

Among the five tested extracts of *Camellia sinensis*, it was found that *Camellia sinensis* methnol extract was most potent in suppressing protein denaturation with an inhibition percentage of 24.62, 43.63 followed by 47.02 at the concentration of 100, 250, 500 µg/ml respectively.

Table 9: Percentage inhibition of protein denaturation exhibited by various extracts of *Camellia sinensis*

Group	% inhibition
Normal group	65.2
Standard group	50.25
Methanol extract(100 µg/ml)	24.62
Methanol extract(250 µg/ml)	43.06
Methanol extract(500 µg/ml)	47.03

The *camellia sinensis* was identified collected and shade dried. The organoleptic properties of *Camellia Sinensis* plant material are tabulated in table.1. The shade dried plant material was converted into moderately coarse powder. Ash content value of *camellia sinensis* was found 7.52% w/w, 5.01% w/w, 6.01% w/w and 5.2% w/w of methanol extract, water extract, Chloroform extract and pet ether extract respectively. Acid insoluble ash was found to be 2.30% w/w, 3.18% w/w, 2.25% w/w and 1.3% w/w of methanol extract, water extract, Chloroform extract and pet ether extract respectively. Water soluble ash was found to be 6.20%, 16.2%, 17.3% and 13.23% of methanol extract, water extract, Chloroform extract and pet ether extract respectively. Loss on drying was found to be 5.50 % w/w, 4.12 % w/w, 3.12 % w/w and 2.13 % w/w of methanol extract, water extract, Chloroform extract and pet ether extract respectively. Foreign matter was found to be 0.5% w/w, 0.1% w/w, 1.15% w/w and 0.63 % of methanol extract, water extract, Chloroform extract and pet ether extract respectively. TLC analysis, different absorbing bands were observed under UV light (254 nm and 336 nm). The various extract obtained after the extraction were subjected to phytochemical screening for determination of various class of pharmaceutical constituents present in *camellia sinensis*. Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Steroids & terpenoids, Carbohydrates were found positive in camellia sinensis.

CONCLUSION

Our research work were investigated phytochemical parameters of leaf of *Camellia sinensis*, by using loss of drying, ash value, acid soluble ash and water and alcohol soluble extractive values, qualitative and quantitative test. Preliminary phytochemical screening test was performed using water, methanol, chloroform and petroleum ether. The results of phytochemical revealed the highest phyto compounds were present in methanol, water, chloroform and petroleum ether and all the pharmacognostic parameters under the limit as per Indian Pharmacopoeia. Anti-inflammatory activity of *Camellia sinensis* extracts was investigated and determined by using *In-vitro* percentage inhibition of protein denaturation method. The evaluation results of five tested different extracts of *Camellia sinensis* on percentage inhibition of protein denaturation method indicated that *Camellia sinensis* methnol extract was exhibited potent protein suppressing denaturation property. The percentage inhibition of protein denaturation was found to be 24.62, 43.63 followed by 47.02 at the concentration of 100, 250, 500 µg/ml respectively. Results of percentage inhibition of protein denaturation represented that methanolic extract having important source of bioactive components and need to identity the quality and purity of the plant material in future studies.

ACKNOWLEDGEMENT

I appreciate the administration of this excellent professional institution for giving the top lab facilities I needed to do my study.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

REFERENCES

1. Ody, P. (1993). The Complete Guide to Medicinal Herbs (1st ed.). Dorling Kindersley.
2. Lichterman, B. L. (2004). "Aspirin: The Story of a Wonder Drug". British Medical Journal. 329 (7479): 1408. doi:10.1136/bmj.329.7479.1408. PMC 535471.
3. Gershenzon J, Ullah C (2022). "Plants protect themselves from herbivores by optimizing the distribution of chemical defenses". Proc Natl Acad Sci USA. 119 (4). doi:10.1073/pnas.2120277119. PMC 8794845. PMID 35084361.
4. Ahn, K. (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs". BMB Reports. 50 (3): 111-116. doi:10.5483/BMBRep.2017.50.3.221. PMC 5422022. PMID 27998396.
5. ITIS Standard Report Page Camellia Sinensis retrieved 2009-03-28.
6. Preedy, V.R. (2013). Tea in Health and Disease Prevention. Elsevier Science: 199-200. ISBN 978-0-12-384937-3.
7. Golender L (2016), "Botanics" The first edition of Linnaeus's Species Plantarum published in 1753 suggested calling the tea plant Thea sinensis.
8. Medzhitov R. Inflammation (2010): new adventures of an old flame. Cell. 2010; 140:771-776.
9. Zhou Y, Hong Y, Huang H. Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-Nephritis Rat via Downregulation of NF-κB Signaling Pathway. Kidney and Blood Pressure Res. 2016; 41:901-910.
10. Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. (2007)Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clin Exp Immunol. 147:227-235.
11. Takeuchi O, Akira S. (2010). Pattern Recognition Receptors and Inflammation. Cell. 140:805-820.
12. Jabbour HN, Sales KJ, Catalano RD, Norman JE. (2009). Inflammatory pathways in female reproductive health and disease. Reprod. 138:903-919.
13. Gudkov AV, Komarova EA.(2016). p53 and the Carcinogenicity of Chronic Inflammation. CSH Perspect Med. 6(11): a026161.
14. Lawrence T. (2009). The Nuclear Factor NF-κB Pathway in Inflammation. CSH Perspect Biol. doi: 10.1101/cshperspect.a001651.
15. Libby P. (2007). Inflammatory mechanisms: the molecular basis of inflammation and disease. Nutr Rev. 65:S140-S146.
16. Brusselle G, Bracke K. (2014). Targeting immune pathways for therapy in asthma and chronic obstructive pulmonary disease. Annals American Thoracic Society. 11:S322-S328.
17. Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of microbiology & biology education, 15(1), 45-46.

CITATION OF THIS ARTICLE

Swarup J. Chattarjee, Rajasekaran S. Assessment of Anti-Inflammatory Effect of *Camellia sinensis* using *in-vitro* study Bull. Env.Pharmacol. Life Sci. Vol 12[5] April 2023: 185-194.