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Phytochemical analysis, anti-oxidant activity, anti-microbial activity of leaves of *Camellia sinensis* (theaceae family) (white tea)

Khushboo Tewani

Department of Pharmaceutics, Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh, India

*Corresponding author email: Khushi.791tewani@gmail.com

Dr. Monika

Department of Pharmaceutics, Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh, India

Email: madhra1282@gmail.com

Abstract---White tea, also known as *Camellia Sinensis* (Theaceae family), is a lesser-known variety of the world-famous *Camellia Sinensis* plant and beverage, despite its possible health advantages, cheap cost, and energizing effects. In order to prevent the breakdown of polyphenols, which prevents chlorophyll from combining with the leaf buds, young tea shoots are kept away of direct sunlight. The goal of herbal medicine research & development is to enhance the quality and safety of natural products. Analyses of phytochemicals and physicochemical were performed. Different concentrations of white tea have been studied microbially. Tannings, flavonoids, glycosides, and saponins were found in the materials during a phytochemical investigation. In the ethanolic extract of white tea leaves, polyphenol content is 36.25 percent, with catechins making up 19.10 percent and tannins making up 17.52 percent of that. Catechins include 7.98 percent EGCG as a derivative. The phytochemical properties reported in this study could be included in the pharmacopoeial standard, which could help standardize it.

Keywords---phytochemical, pharmacognostic, physicochemical, white tea, theaceae.

Introduction

Indonesia is a country with a large Muslim population. Herbal health medicine development is predicted to pick up steam right now. Preventing illness and

curing the sick are among the many ways herbal medicine is predicted to contribute to public health improvement (curative). Fortunately, the country's natural resources, which include more than 30,000 kinds of medicinal plants, contribute to this improvement. Plants that have been utilised for therapeutic purposes for thousands of years include 6,000 types of medicinal plants. Tea is a most popular and widely supplied beverage obtained from *Camellia Sinensis* plant (Figure.1) (1)(2). It is classified within the family Theaceae. Depending on fermentation process, Tea is classified as green, black, white, yellow, oolong, Pu-erh etc (3).

It is a safe alternative to espresso and alcoholic beverages, since it includes no additional chemicals, is completely natural, does not contain energy, fat, or caffeine, and is completely free of calories. For countries like India, Sri Lanka, China, Turkey, Japan, and Russia, the US Food and Drug Administration (USFDA) declares tea to be a safe beverage for human consumption.(4). In early spring, Once a year, White Tea is harvested. Silky white feathers covering unripe leaves and buds are the roots of the name of White Tea(5). It has a sweet taste that is delicate and mild(6)(7). Tea leaves are evaporated and dried directly after being collected to avoid oxidation(7). The majority of white tea are produced in tea plantation located in the south of china, in the province of Fujian(5).There are four big white tea varieties available from China; Silver Needle, White Peony Long Life Eyebrows and Tribute eyebrow. Another White Tea comes from India's Darjeeling region(8). White tea is unfermented tea produced using youthful *Camellia Sinensis* shoots. Ordinarily it is protected from daylight to forestall the corruption of Polyphenols. It is occasional yield with exceptional wellbeing and tangible advantages and lower levels of caffeine than Green Tea (7).



Figure 1. Dried White Leaves

Composition of *Camellia Sinensis* (White Tea)

Aromas, Amino Acids, Lignin, Polysaccharides, and Methylxantins (Caffeine, Theophylline, and Methylxanthins) are all present in White Tea (9). Isolated white tea polysaccharides are water-soluble polysaccharides found in White Tea. Flavanols, catechins, anthocyanidins, flavones, and isoflavanoids are all kinds of flavanoids that are crucial for human health and are found in nature in around 4000 distinct forms (11). In the phenolic family, catechins such as EGCG, ECG,

and EGC are all examples of the catechins found in green tea (Al-Sayed and Abdel-Daim 2018). Amount of ECGC in White tea leaves ranges from 50 to 80 percent. A total of eight catechins are found in the bioactive components, including four different types of Epicatechin (EGC), four different types of Epicatechin Gallates (ECG), four different types of Epicatechin (EC), and four different types of Epigallocatechin Gallates (EGCG), and four different types of Transcatechin (GC), all of which are derived from Epicatechin (12). (Figure.2)

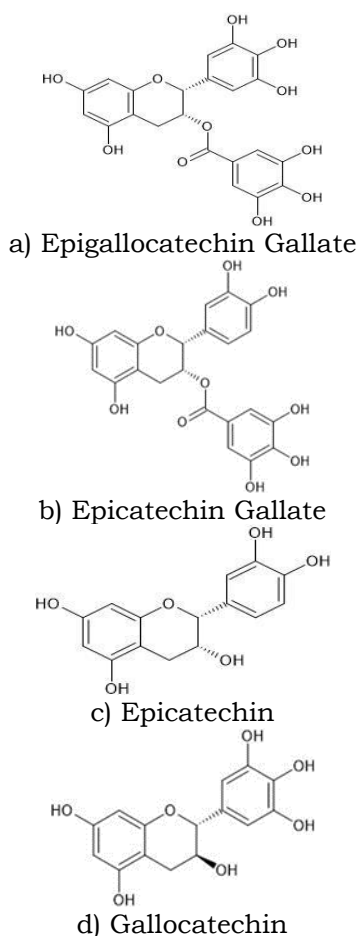


Figure 2. Phenolic compounds of *Camellia Sinensis*

Benefits of *Camellia Sinensis* (White Tea)

Immunity boosting agent

Medicinal Natural sources itself act as an immune-modulating agents and alternative to 'conventional chemotherapy'. It also repairs the innate immune response by activating the host defence mechanism(13).

Rich in Antioxidants

White tea is filled with catechins, a former of polyphenol(14). Polyphenols is a plant based molecule which act as an antioxidants. antioxidants protects the cells from damage by compounds called free radicals. Only white tea can combat free radicals, which cause ageing, chronic inflammation, a weakened immune system, and a number of diseases. (15).

Reducing the risk of heart disease

White tea contains Polyphenols which, in several ways, help reduce the risk of heart disease(16). It also help to relax the blood vessels and boost up the immunity. it prevents the bad LDL cholesterol from becoming oxidized which cause major risk(17)(18). Drink white tea more than 3 days lowers the risk of heart disease up to 21%(19).

Helps in losing weight

White tea extract stimulates the breakdown of fat and also prevent the formation of new fat cells. White tea also boost up the metabolism by an extra 4-5% (70-100 calories per day)(20).

Has compounds that can fight with cancer

White tea may have anticancer effects. According to test tube study White tea has extracts that can trigger the cell death of several lung cancer(21).

Lower the risk of Insulin Resistance

Individuals who avoid reacting to insulin will lead to insulin resistance due to high sugar consumption[53]. According to an animal study, White tea which contains EGCG and other Polyphenols enhance the effect of insulin and prevents elevated blood sugar level(22).

Work against Osteoporosis

Osteoporosis is a condition in which bone gets hollow and porous. Approx 44 million Americans over the age of 50 affected and leads to fracture. there are two factors free radicals and chronic inflammation which accelerates the osteoporosis and suppress the cells that exhibits the bone growth and promotes the cell that breakdown the bones(23).

Helps in the treatment of Parkinson's and Alzheimer's diseases

White tea contains Polyphenols EGCG, which helps to reduce the risk of developing Parkinson and Alzheimer disease. EGCG prevents Proteins from inappropriately folding and clumping together. Inflammation and nerve damage in the brain are caused by misfolded and clumped protein. An analysis of 8 studies showed that 5600 people who drank white tea had a 15% lower risk of Parkinson's disease than those who did not, and 26 studies found that 52500 people who drank this tea had a 35% lower risk of brain disorders like Alzheimer's disease.(24). (Figure.3)

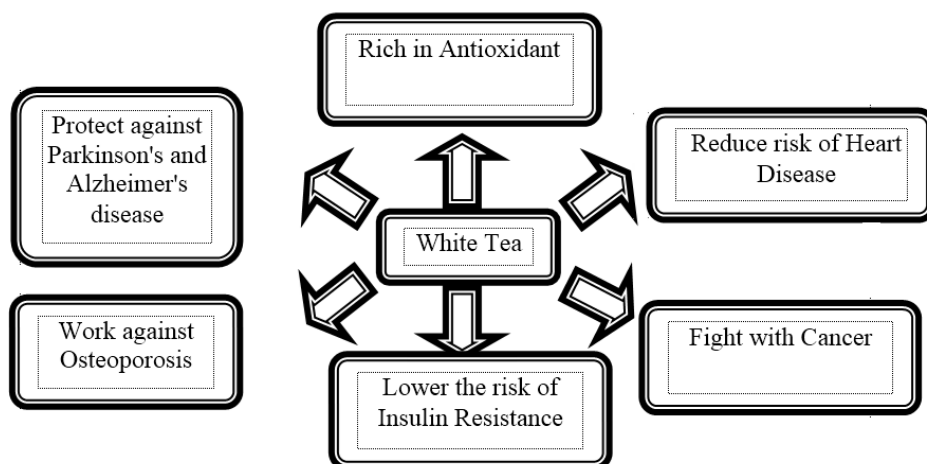


Figure 3. Benefits of White Tea

Material and Methods

Preparation of Material

In Shillong, India, Kashayam Blends provided white tea leaves that had been filtered, collected, and then sun-dried. To further reduce the size of the tea leaves, a drier is used. Using a food processor, white tea leaves are ground into a fine powder.

Preparation of White Tea Extract

Using the reflux method, white tea leaves were removed for more than three hours at 60 C using 70 percent ethanol as a dissolvable and 70 percent ethanol. Supernatant was collected and transferred to the volumetric carafe for two further refluxes before it was filtered and used. The ethanol extract was evaporated using a vacuum rotatory evaporator.

Identification of Raw Material

The process of identifying natural substances is completed by paying attention to their organoleptic, visual, and small characteristics. When it comes to white tea, there is no better way to prove its authenticity than by comparing it to other types

of tea leaves in terms of length and breadth as well as plant size and shade. A Microscope IX70 was used to determine that the dried colour was not permanent. White tea leaves that have been cut in half lengthwise and then powdered are used to create micro-ids. Electron microscopy may also be used to identify powdered compounds that haven't been polished (FE-SEM).

Extract Characterization

The parameters of the extract were determined by analysing its moisture content, ash content, acid-insoluble ash content, water-soluble extract content, and ethanol-soluble extract content. In compliance with WHO guidelines, this extract's constituents were identified. Using aquadest, we extracted 50 g of dry tea and macerated it for 10 minutes at 60^o C in a water shower shaker with a material-to-dissolvable ratio of 1:10 (w/v). Once the design had been thought out, a spinning vacuum evaporator was employed to create a thick fluid. It was then freeze-dried and kept in a refrigerator for subsequent examination after being taken from an aqueous solution. In the past, this approach has been put to use several times. (Table.1)(25)

Phytochemical Analysis

Chemicals are screened and significant constituents are found in phytochemical analysis. The presence of alkaloids and tannins, flavonoids, saponins, terpenoids, glycosides, and anthraquinones in the plant samples was examined in accordance with WHO recommendations and Harborne's protocols. Using gallic acid as a reference, researchers were able to compute the proportion of gallic acid equivalents in polyphenol content. To conduct this experiment, a 100-ml flask is filled with a 10 g sample, which has been dispersed in 50 ml of distilled water, and then sonicated for 10 minutes. Next, a millilitre of the sample is removed and placed in an analytical flask for testing and research purposes. An first 5 mL of Folin Ciocalteu 10 percent reagent was added to the mixture and let to stand for 3-8 minutes. The combination was ready for testing after two hours of dark incubation. If you have a spectrophotometer, you may also measure the solution's absorbance at 750nm. The complete tannin content was resolved utilizing the spectrophotometry procedure. 1 g of material is gauged and put in a 100 ml carafe, which is then weakened with refined water. An aliquot of one milliliter was put in a flagon and weakened with 75 milliliters of refined water. 5 mL Folin-denis reagent, 10 mL soaked sodium carbonate arrangement, and 100 mL refined water were added and homogenized. The absorbance of this blend was estimated at 760 nm following 30 minutes of hatching. (Table.2)(26)

Determination of Catechin and ECGC

A spectrophotometer was used to quantify the total catechin content at 210 nm. The researchers used catechin equivalents as a percentage of total catechin equivalents as a baseline. At pH = 4.00, reverse phase HPLC was used to perform ECGC assays using an isocratic mobile phase consisting of orthophosphate 0.1 percent (w/w) at pH 4.00 and flow rate of 1 mL/min for the reverse phase HPLC assays.

Chromatography Analysis

To do the chromatographic analysis, we used the TLC method. For the stationary phase, chromatographic precoated silica gel plates were loaded with ten microliters of white tea ethanolic extract and five microliters of a catechins standard (Merck, TLC grade). In a glass chamber with twin troughs, a mobile phase of acetone, toluene, and formic acid (4:5:1 v/v) was employed to create chromatograms. Afterward, the plates were taken out of the solvent and allowed to dry. An iron (III) chloride solution of 1 percent sprayed the plate before iron (III) chloride was applied (FeCl₃). The plate was then inspected under visible (white) and ultraviolet (254 nm) light a few minutes later. To illustrate, the retention factor of a combination may be used to estimate the maximum distance that each component can travel (R_f). The formula was used to calculate the values for each site.

$$R_f = \frac{\text{Distance from starting point to the centre of the spot}}{\text{Distance from the starting point to the solvent front}}$$

Microbiological Analysis

Zone of Inhibition was measured using the Well diffusion method, as well as harmful microbiological contamination such as *E. coli*, *S. Aureus* and *Pseudomonas aeruginosa*. *Escherichia coli*, *Staphylococcus Aureus*, *Pseudomonas aeruginosa* were clinically confined and sympathetically given by Dr. Neeraj Verma from Center of Nanoscience, Foresight Biotech Private Limited, JNU, Delhi. Against microbial movement was finished by Well Diffusion Plate Method. Microscopic organisms was initially filled in Nutrient Broth (Brain Heart Extract). The microscopic organisms were then immunized utilizing sterile swabs on isolated Trypticase Yeast-Extract Cysteine agar (TYC agar) (Sigma-Aldrich, US). On the TYC agar, 6-millimeter distance across well were punched and pour the tea separate as indicated by various focuses in it. Stock centralization of medications are 10,20,50,100 mg/ml. These centralizations of 20 microliter of medication fill the well and afterward hatch over night at 37°C. After short-term hatching, for against bacterial movement agar plate were analyzed by estimating the zone of restraint. (Table.3)

Determination of Anti-oxidant Activity DPPH

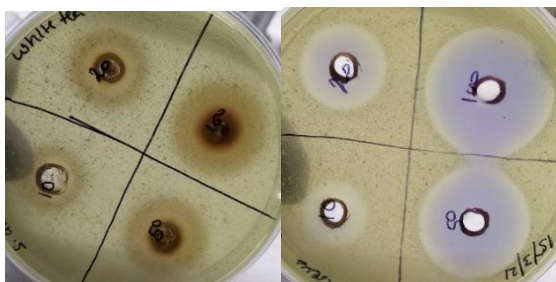
White tea's capacity to neutralise DPPH free radicals was evaluated using a modified version of the Ye and Huang(28) method. Samples were diluted with either 0.2 millilitres of previously diluted sample infusion or a particular amount of EGCG (>95 percent) before the DPPH solution was added. For 30 minutes, the mixture was maintained at 25°C in the dark. The 517 nm absorbance must be known. Eq. 1 was used to compute the DPPH scavenging activity (1). (28)

$$\text{DPPH SA (\%)} = (1 - A_s / A_c) \times 100 \quad \dots\dots (1)$$

While DPPH SA refers to DPPH scavenging activity, A_s and A_c stand for sample and control absorbance, respectively. (Table.4)

Result

The characterization of organoleptic observations of *Camellia Sinensis* leaves, including shape, smell, colour, and taste. White tea, like other teas, has a distinct scent and astringent flavour. The macroscopic study of the leaf is comprised of botanical observations. The microscopic investigation, on the other hand, was carried out on a cross-section of a leaf or a specific part fragment of leaf powder. The goal of this observation is to look at specific portions of the organs of the plants that were employed. White tea leaf extracts were produced using a 70% ethanol solution and a reflux extraction method at 60°C. The extract has a yield of 57.08 percent. The crude extract was red-brownish in colour and had a characteristic tea scent. Physicochemical and phytochemical analysis were used to examine the extract. TLC profile is one of the ways for determining the presence of a marker component in an extract. After post-derivatization with FeCl₃ 1 percent, the TLC profiles of white tea extract and catechin standard were obtained under UV 254 light. Isolated compound with specified R_f values (R_f= 0.60) is represented by a distinct TLC spot on a silica gel plate. Moisture, ash, acid insoluble ash, water-soluble extract, and ethanol-soluble extract were all tested as part of the extract's physicochemical analysis (Table 1). The extract contained alkaloids, flavonoids, tannins, glycosides, and saponins, according to phytochemical examination. Terpene/steroid and anthraquinone yielded poor results. The polyphenol content of the ethanolic extract of white tea leaves is 36.25%, with the highest concentration of catechins at 19.10% and tannins at 17.52%. EGCG, a derivative of catechins, has a 7.98 % content.



(a) Zone of Inhibition in *Staphylococcus Aureus* (b) Diffusion of Antibiotic (Ciprofloxacin)



(a) Zone of inhibition in *Escherichia Coli* (b) Diffusion of Antibiotic (Ciprofloxacin)



(a) (b)

(a) Zone of inhibition in *Pseudomonas aeruginosa* (b) Diffusion of Antibiotic (Ciprofloxacin)

Figure 4. Zone of Inhibition of White tea against *Staphylococcus Aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*

Table 1
Characterization of ethanolic extract of white tea leaves (*Camellia Sinensis*)

No.	Physico-Chemical Parameter	Result (%)
1.	Acid Insoluble ash content	0.15
2.	Ash Content	6.24
3.	Moisture Content	15.98
4.	Alcohol soluble extract content	33.35
5.	Water-soluble extract content	16.99

Table 2
Phytochemical Analysis of White Tea Leaves Ethanolic Extract

No.	Phytochemical Parameter	Result
1	Alkaloids	+
2	Terpene	-
3	Tannins	+
4	Anthraquinone	-
5	Saponin	+
6	Flavonoids	+
7	Glycosides	+
8	Total Polyphenol	36.25%
9	EGCG content	7.98%
10	Total Catechin	19.10%
11	Total Tannin	17.52%

Table 3
Zone of Inhibition of White tea against *Staphylococcus Aureus*, *Escherichia Coli*,
Pseudomonas aeruginosa

S. No.	Bacteria	Concentration (stock of 10, 20, 50, 100 in 1ml)	Zone of Inhibition (White Tea)in (mm)	Zone of Inhibition (Antibiotic) in (mm)
1	<i>Staphylococcus Aureus</i>	10mg- 50µl	15.25	14.5
2	<i>Staphylococcus Aureus</i>	20mg- 50µl	18.5	16.5
3	<i>Staphylococcus Aureus</i>	50mg- 50µl	23.25	20.25
4	<i>Staphylococcus Aureus</i>	100mg- 50µl	27	25.5
5	<i>Escherichia Coli</i>	10mg- 50µl	13	16.25
6	<i>Escherichia Coli</i>	20mg- 50µl	13.75	18.25
7	<i>Escherichia Coli</i>	50mg- 50µl	15	22.75
8	<i>Escherichia Coli</i>	100mg- 50µl	16.75	25.75
9	<i>Pseudomonas aeruginosa</i>	10mg- 50µl	16	12
10	<i>Pseudomonas aeruginosa</i>	20mg- 50µl	17.75	13.25
11	<i>Pseudomonas aeruginosa</i>	50mg- 50µl	19	16
12	<i>Pseudomonas aeruginosa</i>	100mg- 50µl	21.5	18.25

Table 4
DPPH of White Tea

Sample Concentration (µg/ml)	Antioxidant capacity scavenging DPPH (% RSA) of White Tea
1000	93.01 ± 0.08d
1250	93.87 ± 0.19d
1500	94.00 ± 0.09c

Discussion

Bioactive components are what determine the potency of herbal medications. There are several advantages of TLC fingerprinting for identifying and certifying

bioactive chemicals. A perfect match was found when comparing the catechin Rf values to those in the white tea leaf extract's TLC spots. It was concluded that catechins and other phenolic compounds were the key determinants of astringency because of the higher concentration of catechins and other phenolic compounds. Tea leaves processed in the traditional manner without fermentation contain higher concentrations of polyphenols, mainly catechin derivatives; this is because of the way the leaves are dried. Polyphenol, catechin, and EGCG concentrations were all shown to be healthy.

White tea leaf extract includes alkaloids, flavonoids, tannins, and saponins, according to phytochemical research. White tea, like green tea, has a wide range of health-promoting bioactive (polyphenols, caffeine, theogallin, gallic acid, theaflavins, glycoside flavanols, and catechins). Compared to green tea, white tea has a higher concentration of catechins, such as EGC, EGCG, ECG and EGC. White tea leaf extract's low theaflavin and thearubigin concentration and high catechin content have been connected to the tea's colour (a polyphenol oxidase oxidation product). Each type of tea has a unique composition that is determined by the tea plant itself, regardless of how it is processed, where it is cultivated, or how it grows over time (29).

When it comes to the health of your body, polyphenols found in tea may have a favourable effect. In several investigations, white tea was shown to be a more effective inhibitor of pancreatic lipase than green tea. According to a recent research, white tea leaf extract reduced adipogenesis and increased lipolytic activity. When it comes to treating diabetes, the ethanolic extract of white tea leaves has been shown to be an inhibitor of α -glucosidase, as well as α -amylase and dipeptidyl-peptidase IV (30)

Conclusion

White tea leaves from *Camellia Sinensis* have pharmacognostic properties that may be employed in this research to demonstrate their value. Identifying the pharmacognostic and phytochemical properties of white tea leaf extract and fingerprinting the results would aid in future pharmaceutical production.

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