RJC

RASĀYAN *J. Chem.*
Vol. 17 | No. 2 |417-424| April - June | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

PHYTOCHEMICAL, ANTIMICROBIAL, ANTIOXIDANT, AND CATECHIN ANALYSIS OF GREEN TEA (*Camellia sinensis var assamica*) FROM NORTH SUMATERA, INDONESIA

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ABSTRACT

The taste of green tea is different and unique; factors that contribute to this include the type of picking method, leaf age, variety, and clones. The researchers used the disc method for antimicrobial testing, the DPPH method for antioxidant testing with a visible spectrophotometer set to 515 nm wavelength, the HPLC method for catechin content analysis, and phytochemical screening as a qualitative test. The results of this study's phytochemical screening tests revealed that green tea was positive for terpenoids with Liebermann Bouchard reagent, flavonoids, and tannins with $FeCl_3$ reagent, and good inhibition zones for pathogenic bacteria of *Staphylococcus aureus, Streptococcus mutans, and Salmonella typh*i. The outcomes of the negative control, 5%, 10%, and 15% green tea ethanol extract against the following microorganisms were: 0; 4,3; 6,35; 8,05 mm for *Staphylococcus aureus*, 0; 5,9; 6,95; 8,55 mm for *Streptococcus mutans*, and 0; 3,45; 4,4; 6,45 mm for *Salmonella typhi*. Results indicated that *Salmonella typhi* and *Staphylococcus aureus* were more effectively inhibited by green tea ethanol extract than *Streptococcus mutans*. Antioxidants demonstrated IC_{50} of 33.33 indicating a very significant antioxidant activity in the test. determined using a visible spectrophotometer. The HPLC test yielded a catechin content of 16.9%.

Keywords: Sidamanik, green tea, disc method, DPPH, HPLC, IC_{50}.

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INTRODUCTION

Camellia sinensis (tea plant), which yields tea leaves, is well-known for its many health benefits when consumed as a beverage. The "Camellia" was taken from Latin, Rey Georg Kamel who is a pharmacist and missionary. Carl Linnaeus had chosen his name for the tea genus in 1753 in honor of Kamel's contributions to botany, while "sinensis" means "came from China" in Latin.[1] Some researchers said that Indonesian tea has a lot of catechins. The determination of flavor and scent is significantly influenced by catechins. Catechins are colorless, water-soluble chemicals that give brewed tea its bitter and astringent qualities. This is the most important ingredient in tea leaves since it has the potential to impact the tea's quality.[2] Based on the procedure, tea is divided into four categories: oolong tea (partially fermented), green tea (fermented, dried, and withered, with a major amount of Epigallocatechin gallate (EGCG), and white tea (non-fermented, withered, and mostly composed of polyphenols and fluoride). mostly dimethyl EGCG) and black tea, whose primary component is flavin when it has fully fermented.[3] Several additional variables, such as the tea's geographic origin, leaf age, plucking method, varietals, and clones, can further impact its quality.[2] Due to the rarity of recorded harmful effects on humans and animals, brewed teas and tea-based products are typically regarded as safe for users. Although certain tea samples contain heavy metals, pesticide residues, and mycotoxins, tea drinking can still be advised to the general public for the management of chronic illnesses.

Tea can also be made into drinks, useful meals, and pharmaceuticals.[4] Instead of going through a fermentation process, green tea leaves go through a drying and evaporation process.[5] Catechins as the main polyphenols in green tea. We can observe the catechin derivatives in the Table-1.

http://doi.org/10.31788/RJC.2024.1728738

Table-1: Catechins Type and Their Levels in Green Tea[6]

Catechin Derivatives	% of solid extract
Epigallocatechin gallate (EGCG)	20.3
Epicatechin gallate (ECG)	5.2
Epigallocatechin (EGC)	8.4
Epicatechin (EC)	2.0

Catechins, specifically EGCG, ECG, EGC, and EC are typically found in green tea. The primary catechin in green tea, EGCG, has been extensively researched and shown to have positive health effects.[4, 7] Several health issues are defended against using green tea. Green tea's bioactive components, which are responsible for its nutritional, immunological, pharmacological, and physiological properties, have the potential to treat and prevent a wide range of illnesses in both humans and animals.[8] Green tea is a safe, non-toxic, and fairly priced beverage that has been demonstrated to have antimicrobial effects on several harmful microbes.[9] Experimental research on cells, animals, and humans has proven that EGCG, the main component in green tea, has anti-inflammatory qualities.

These genes, as well as the protein production of inflammatory cytokines and enzymes linked to inflammation, are suppressed by EGCG and green tea.[10] The functional group, the surrounding conditions, and the stability of phenolic oxygen radicals all affect how effective tea polyphenols are as antioxidants in scavenging free radicals. Tea polyphenols work in concert with other nutrients to scavenge free radicals, reduce oxidation through metal ion chelation, increase the enzyme activity of antioxidants, and inhibit lipid peroxidation once they are inside the body.[11] EGCG contained in green tea predominantly has strong antioxidant activity.[12]

Green tea extract has been investigated for its antibacterial activity. One of them is to inhibit the growth of *Streptococcus mutans* as a bacterium found in the mouth.[13] Antibacterial properties of green tea have also been widely studied against *Streptococcus aureus* which is resistant to several antibiotics.[14, 15,16] The antibacterial properties of green tea against *Salmonella typhi* have also been widely studied. [17, 18, 19] This study aimed to gather data on the presence of secondary metabolites and their activities in green tea from Sidamanik, North Sumatera Province, Indonesia. The quality of green tea is now being improved through additional research. Since there has been relatively little research done on Sidamanik green tea, the authors hope to further the field of study by looking at the catechin as a major and its activities.

EXPERIMENTAL

Sampling

The tea leaves utilized in this study came from the PTPN IV, Sidamanik tea estate in the Simalungun Regency, North Sumatra 21171, Indonesia, Ambarisan, Sidamanik District.

Fig.-1: Map of the PTPN IV Sidamanik, North Sumatera, Indonesia, 2°51'35.5"N 98°55'02.0" E. (Google Maps)

Tea Determination

The fresh plants (leaves and branches) from the Sidamanik tea plantation were sent to the Herbarium Laboratory of Biology to determine their taxonomy classification, at the University of Sumatera Utara, Medan, Indonesia.

Green Tea Leaf Phytochemical Screening

A total of 100 g of green tea leaf powder was taken and then put into an Erlenmeyer tube. Ethanol as solvent was added and covered with seal wrap, soaked for 24h, and then the filtrate was taken. The filtrate was

then placed in a test tube with as many as 5 drops each and tested on the reagent, observed the changes that occurred, and the results were recorded.[20]

Green Tea Leaf Extraction
Dried green tea was extracted by maceration using 96% ethanol solvent for 24 h. Subsequently, the filtrate is extracted, and concentrated to yield a concentrated extract, and the remaining ethanol is evaporated until it is no longer present, leaving behind a thick extract.

Green Tea Leaf Antimicrobial Activity Test
Following that, different extract concentrations of 5, 10, and 15% were added to the thick extract by diluting it with distilled water.

Bacterial Culture Preparation
A round needle was used to remove the bacterial colonies (*S. aureus*, *S. mutans*, and *S. typhi*), sterilize, and then transplant the bacterial colonies onto the surface of the nutritional medium. After that, it was scraped, turned, and then incubated for 24h at 37°C.[21]

Bacterial Inoculation Production
S. aureus, S. mutans, and *S. typhi* bacterial colonies were isolated from the culture stock using sterile eyelets. The colonies were then suspended in a tube with 10 mL of nutritional medium and cultured at 37°C for 24h, or until the turbidity was similar to that of Mc. Farland.[21]

Antibacterial Activity Test
Before adding the nutrient agar medium and shaking the petri dish on a countertop to integrate the medium and bacterial suspension, a sterile petri dish should be filled with up to 0.1 mL of bacterial inoculum (*S. aureus*, *S. mutans*, and *S. typhi*). Disc papers were provided for every test solution concentration. Next, set it down on a level, medium-sized surface. The sample was incubated for 18 to 24h at 37°C. A caliper was used to measure the diameter of the pressure zone that developed around the paper disc.[21]

Green Tea Leaf Antioxidant Activity Test
The tests carried out were antioxidant activity testing utilizing the DPPH method where antioxidant activity may be seen from the value of Inhibition Concentration 50 (IC_{50}) (half maximal inhibitory concentration). The following formula can be used to determine the percentage of inhibitory activity against DPPH radicals from each concentration of sample solutions: % Inhibition = control absorbance − sample absorbance divided by control absorbance x 100%.[22]

Green Tea Leaf Catechin Test
Chromatographic Conditions
Two solvents were eluted gradient at a flow rate of mL/min (min 21). Water:acetonitrile: formic acid (94.7:4.3:1 v/v) and water:acetonitrile: formic acid (49.5:49.5:1 v/v) were the solvent compositions that were utilized. The composition's mobile phase began with 90% solvent A and 10% solvent B. Every sample was placed in a microfilter before being injected.[23,24]

Sample Condition
The concentrations of standard catechins at 25, 50, 75, and 100 µg/mL were first determined. Subsequently, it was found that green tea always included catechins. Green tea samples with labels were given out. With a small change, the samples were extracted: at 40 min at 24^0C, and continuous stirring was required for the extraction of green tea extract (0.5 g in 100 mL) of acetonitrile: water (1: 1 v/v). This solution was diluted and injected for HPLC.[25]

RESULTS AND DISCUSSION

Tea Determination
The fresh plants (leaves and branches) that were sent to the Herbarium Laboratory of Biology, University of Sumatera Utara, Medan, Indonesia, are *Camellia sinensis var assamica.* The variety *assamica* tea plant is characterized as an 8–12 meters tall, towering tree with numerous branches. The leaves have a glossy surface, light green color, and measure 15-20 cm in length.

Phytochemical Screening

To identify the precise components included in the simple investigated compounds, a preliminary procedure called phytochemical screening was carried out on extracts of *Camellia sinensis var assamica* leaves. The solvent used for extraction is a crucial component of the phytochemical screening process since the solubility of the compounds under study can be impacted by other chemical groups. The following table details the outcome of the phytochemical screening that was done on leaf extracts of *Camellia sinensis var assamica.*

Table-2: Phytochemical Screening Results

Reagents		Tea Leaves Results
Alkaloidd	Bouchardat	-
	Meyer	-
Flavonoid	$FeCl_3$	+
	Mg.HCl	-
	H_2SO_4	-
Terpenoid	Liebermann-Bouchard	+
	Salkowsky	-
Steroid	Liebermann-Bouchard	+
	Salkowsky	-
Saponin	Distilled water	+
Tannin	$FeCl_3$	+

The information "+" indicated there was a compound and "-" indicated there was no compound formed when reagents of phytochemical compounds. From the table above, we found that green tea contains flavonoids, tannins, terpenoids, and steroids. If we analyze more activities of plants, we should start to analyze the phytochemical screening of plants because the secondary metabolites in plants determine activities in that plant. [26,27, 28]

Antibacterial Activity Results

Green tea extract's antibacterial against pathogenic bacteria, namely *S. aureus, S mutans,* and *S. typhi* were able to be seen in the pictures below.

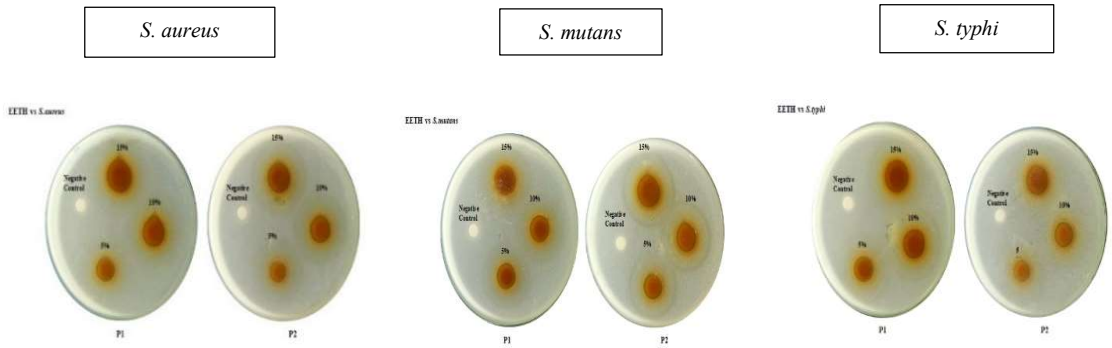

Fig.-2: Green Tea Extract Antibacterial Activity Tests against *S. aureus, S. mutans, and S. typhi*
(P_1: repetition 1, P_2: repetition 2)

A table was created using the images above to help visualize the diameter of the inhibition zone as below.

Table-3: Green Tea Extract Antibacterial Activity Test Results

Green Tea Concentration	Repetitions	Diameter of the inhibition zone (mm)		
		S. aureus	*S. mutans*	*S. typhi*
Negative Control	P_1	6	6	6
	P_2	6	6	6
		0	0	0
15%	P_1	14,5	14,2	11,7
	P_2	13,6	14,9	13,2
		8,05	8,55	6,45

10%	P_1	12,3	12,7	9,2
	P_2	12,4	13,2	11,6
		6,35	6,95	4,4
5%	P_1	10,4	11,8	8,4
	P_2	10,2	12,0	10,5
		4,3	5,9	3,45

From the table above, it is seen that green tea has good activity against pathogenic bacteria *S. aureus, S. mutans,* and *S. typhi*. The outcomes of green tea ethanol extract against *S. aureus* at 5, 10, and 15% negative blank were 0, 4.3, 6.35, 8.05 mm, S. *mutans* were 0, 5.9, 6.95, 8.55 mm, and *S. typhi* were 0, 3.45, 4.4, 6.45 mm. Of the three bacteria, the best inhibitory activity was *S. mutans*, followed *by S. aureus,* and *S. typhi*. For more details, see the diagram below.

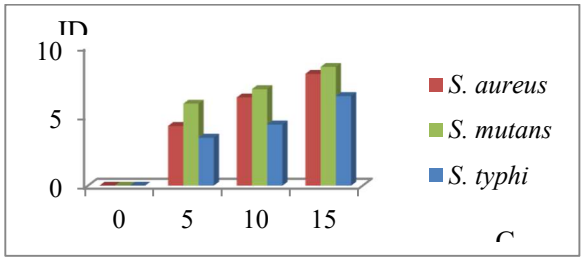

Fig.-3: Green Tea Antibacterial Activity Percentage Diagram

S. mutans is a harmful bacterium that can dwell in the mouth, whereas *S. aureus* and *S. typhi* may live in human intestines. *S. aureus* also exists on the skin and mucous membranes, as well as in the environment. *S. aureus* can cause serious infections by entering the circulation or interior tissues.[29] Oral green tea consumption improved the illness caused by *S. typhi*. [30] The inhibitory activity of *S. mutans* and *S. aureus* was effective against black tea.[31] A test method employing DPPH as free radicals is the most typical technique used to evaluate the antioxidant ability of plants. This objective method is to determine the equivalent concentration parameter that yields a 50% inhibitory concentration as antioxidant activity (IC_{50}). To evaluate the antioxidant activity of an extract's contents, employ a free radical known as DPPH because it can react with materials that produce a hydrogen atom. The unpaired electron in DPPH results in significant absorption at a wavelength between 515 and 517 nm. After the electrons are divided into pairs due to the presence of a free radical catcher, calculate the absorbance drops stoichiometry by counting the number of electrons gathered. Antioxidant chemicals can turn the DPPH solution from purple to yellow.[32] Using a visible spectrophotometer set to 515 nm in wavelength, the data collected can be used to calculate the antioxidant activity of green tea extract, the data obtained are as follows.

Table-4: Green Tea Leaf Ethanol Extract Antioxidant Activity Test

No	Green tea concentration (ppm)	A_1 (Abs)	A_2 (Abs)	A_3 (Abs)	An average	% Inhibitions
1	5	0,199	0,198	0,197	0,198	91,75
2	15	0,178	0,178	0,177	0,178	92,58
3	25	0,154	0,154	0,153	0,154	93,58
4	35	0,118	0,119	0,118	0,118	95,08
5	45	0,101	0,100	0,101	0,101	95,79

Note: A for absorbance

From the table above, the highest % of attenuation in green tea ethanol extract was 95,79 % at 45 ppm. Based on data calculation with y= ax + b of regression formula, we counted the value of IC_{50} as y. The IC_{50} of the Sidamanik green tea ethanol extract was 33.33 meant antioxidant activity was very strong. One measure that's commonly used to evaluate test samples' antioxidant activity is the IC_{50} value. It is determined as the number of antioxidants required to reduce the initial concentration of DPPH by half. Therefore, the IC_{50} value decreases with increasing antioxidant activity.[33] states that an IC_{50} value is extremely strong if it is less than 50 mg/L, strong if it is between 50 and 100 mg/L, weak if it is greater

than 150 mg/L, and 101–150 mg/L. A reliable and popular technique for determining the overall antioxidant capability of natural extracts is the DPPH radical scavenging assay. IC_{50} values are frequently used to present the assay's results. However, the concentration of the DPPH solution used in the process determines the reported IC_{50} value. Finding an IC_{50} value independent of the DPPH concentration was the aim of this study to accurately assess the antioxidant capacity of natural compounds.[34]

Catechin Test of Green Tea Leaf

The results of the analysis of standard catechins at concentrations of 25, 50, 75, and 100 ug/mL are shown in the table, then the regression line is obtained as follows: For details, The diagram can be seen below.

Table-5: Standard Catechins Determination

Green Tea Concentration (ug/mL)	Surface Area
25	218605
50	381760
75	521120
100	699593

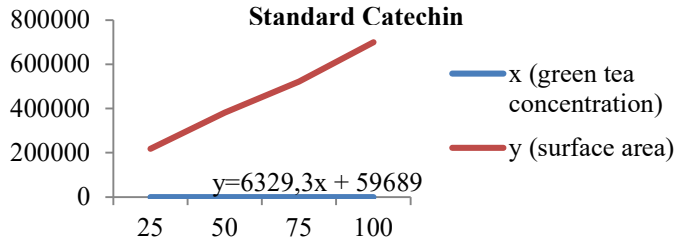

Fig.-4: Standard Catechin Determination

From the catechin standard that has been determined, it can be calculated the value of catechins in green tea leaf samples.

Table-6: Catechins Results by HPLC

ID#	Name	Ret. Time (min)	Area	Height	Conc.
1	Catechin	3.379	599637	75405	131.710

Calculation of Catechin levels in the sample are calculated based on the measured concentration (correlation of the standard curve) which is then converted to % (w/w) units through the sample weight and volume of the extract used (Creg: Measurable concentration based on regression curve (ug/ml); V: Extract Volume (ml); F: Dilution Factor; W: Sample weight (grams); 10000: Convert from ug/g to %).

$$\% \text{ Catechin: } \frac{Creg\ x\ VxF}{W\ x\ 10000}$$

From the results of the analysis using the HPLC method, with a retention time was 3.379 minutes, it was found that the percentage of catechins in green tea was 16.9%. Catechins are the primary antioxidants in green tea. Catechins' antioxidant activity is mostly determined by the number of hydroxyl groups they contain as well as the presence of distinct structural groups. Reactive oxygen and nitrogen species are effectively neutralized by catechins.[34] One tool utilized to assess the catechin content of various samples, including green tea, was the HPLC. It demonstrated favorable outcomes for certain catechin standards and was thought to be accurate in terms of catechin quantification.[24]

CONCLUSION

In conclusion, the taxonomy nomenclature of the plant is *Camellia sinensis var assamica*, and also most of the secondary metabolites (flavonoids, terpenoids, steroids, saponins, and tannins). Using both the disc method, the ethanol extract of green tea leaves exhibits good antibacterial action against *Salmonella typhi, Streptococcus mutans,* and *Staphylococcus aureus* are examples of harmful microorganisms, and very strong antioxidant activity caused IC_{50} as 33.33 by the spectrophotometric approach using DPPH as oxidant

agent. Catechins, a plentiful flavonoid found in green tea leaf extract, were shown to have high concentrations after being evaluated using HPLC obtained 16.9%.

ACKNOWLEDGMENTS

The study was supported by the Ministry of Research, Technology, and Higher Education on September 10, 2021, under the BPI Kemendikbudristek Scholarship (number 1056/J5.2.3. /BPI.06/10/2021), BPPT, and LPDP. The authors express their appreciation to the Ministry for this support.

CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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