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Study of green tea collected from Maheswari tea Estate, Assam

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Abstract
Green tea is obtained from the plant Camellia sinensis belonging to family Theacae. Green tea is a popular neutraceutical as an antioxidant [1]. From ancient times tea is drunk worldwide as a beverage in the form of a decoction because of its several beneficial effect. It was used to detoxify the body [2].This attracted many scientists to work on green tea and discover its therapeutic properties. One of them is its Antioxidant properties. The green tea of Maheswari tea Estate showed some significant results of lowering the blood glucose level to some patients those who were consuming this green tea as an Antioxidant. Considering this, the present article has been focused on the antioxidant aspect of green tea as well as the antidiabetic properties and evaluation of its chemical constituents. This includes the history of this green tea, its pharmacognostical study, chemical constituents. And finally scope of green tea for further research and in designing and formulating drugs of it has been pondered over.

Keywords: Maheswari tea Estate, antioxidant, antidiabetic, chemical constituents

1. Introduction
Green tea is fast becoming a popular drink across all ages due to its perceived health benefits and is fast becoming one of the popular products in the health and wellness segment [3]. The awareness level is especially high in the metro cities or in regions where non-milk tea consumption is more than the national average" [4, 5]. The awareness level about Assam tea is the highest across India followed by Darjeeling tea," the study said. Assam tea is often described as having a malty flavor and a rich, savory aroma. These distinct features are typically attributed to the tea's unique production process. After fresh Assam tea leaves are harvested and withered, they undergo an oxidation process also referred to as fermentation that exposes them to oxygen in a controlled-temperature environment for a designated period of time. Some animal studies suggest that polyphenolic compounds in green tea may help reduce cholesterol and prevent the buildup of plaque in blood vessels.

The various tea types are classified on the basis of their processing, and the associated oxidation and fermentation levels which influence taste and aroma profiles. Green tea is a minimally oxidized and non-fermented tea. Catechin, polyphenols are the primary compounds responsible for the claimed health benefits of green tea, including its antioxidant and anti-inflammatory properties. Caffeine contributes to green tea's stimulant properties, while the amino acid theanine contributes to its relaxing properties. Significant variation of catechin and caffeine content occurs between green tea types depending on environmental growth conditions and processing. Most often, green teas are sourced from the *sinensis* variety of the tea plant while black teas are sourced from the assamica variety [6]. China is the center of origin of green tea production. Today, green tea is produced in over 20 countries in tropical, sub-tropical and temperate regions. It is the most widely consumed beverage after water, due to its health, sensory, stimulant, relaxing and cultural properties. The quality of green tea is impacted by numerous factors involved in cultivation, harvest, processing, storage and preparation that influence chemistry, taste, aroma, morphology and bioactivity of tea leaves. The green tea cultivated in Maheswari Tea Estate showed significant results as an Antioxidant and Antidiabetic.

There is a need of scientific data analysis of its active constituents for its exceptional beneficial effect The Maheswari tea Estate is located in Dhemaji District of Assam There is a river named Gelajan adjoining to this tea garden and during rainy season this tea garden is under water around ninety days under flash water Figure-1 The residual fertile soil may be a reason

for showing som beneficial effect of this green tea.

Fig 1: Maheswari Tea Estate

2. Materials and methods

2.1 Collection of plant material

Green tea leaves from Maheswari Tea Estate, District Dhemaji, Assam was collected and identified at Botanical Survey of India, Shillong, Meghalaya. The collected leaves were washed thoroughly under fresh water and left for drying under shed for 2 weeks. The dried leaves were then powdered in a blender and kept in sealed packets in refrigerator at 4 °C until further use.

2.2 Pharmacognostic studies

Fresh leaves were taken for microscopical studies. Coarse powder (60 #) was used to study physicochemical parameters and phytochemical investigation [7]. For the microscopical studies, transverse sections of leaves were prepared and stained as per standard procedure [7]. Macroscopic study It refers to evaluation through organs of sense and includes the macroscopic appearance, color, odour, taste etc. of the drugs [8].

2.3 Microscopy study

A transverse section of fresh leaf was taken and cleaned. The sample was treated with chloral hydrate solution and different staining reagents and chemicals were used to detect the lignified cells in the cross sections [9]. The section was mounted on slides and studied under Trinocular Research Microscope

2.4 Quantitative estimation

Different physicochemical properties like LOD, PH value, total ash, acid insoluble ash, extractive values were determined using the methods described in the Indian Pharmacopoeia and Ayurvedic Pharmacopoeia [10].

2.5 Qualitative Phytochemical Screening

The aqueous and ethanolic extracts along with other solvent extracts of plant leaf materials were studied for various phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, gums and mucilages, phenols, tannins, reducing sugars, saponins, steroids, tannins and terpenoids by using precipitation and coloration reactions [11].

2.6 Quantitative Phytochemical Screening

Determination of total polyphenol content by folin- ciocalteu method the total polyphenol content (TPC) was determined by spectrophotometer using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The TPC was expressed as mg gallic acid equivalents (TAE)/ g. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to100 µg/ml [12].

2.7 Extraction

300gm of powdered leaf was extracted successively with solvents like petroleum ether, benzene, chloroform, acetone and ethanol respectively in a Soxhlet apparatus [13]. Each solvent extract was then concentrated by distilling off the solvent under reduced pressure. Aqueous extraction of the leaves samples was done by soaking the leaves in sterilized distilled water at approximately 1:10 w/v ratio for 1 h. After soaking, the decoction was brought to boil for 60 min. The liquid extract was separated from the solids by filtration with double-layered muslin cloth and allowed to precipitate. The crude extract was weighed and its percentage yield was recorded. The crude extract was stored at 4 °C until further use (Koh *et al*. 2009)

2.8 Thin layer chromatography

Thin layer chromatography was carried out with the ethanolic extract and maximum spots been separated on precoated silicagel G TLC plate with trial and error methods [7].

2.9 Physicochemical properties

Physicochemical parameters were determined as per guidelines of WHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility of the extract in different solvents, melting point, boiling point, pH, heavy metal analysis, petroleum ether soluble extractive, alcohol soluble extractive and water soluble extractive values were determined [14].

3. Result and observation

The Green tea leaf was investigated in a systematic way covering pharmacognostical, phytochemical, and physicochemical aspects to rationalize its use as a tea of therapeutic importance.

3.1 Macroscopic characteristics

Table 1: (Macroscopic study results)

Characters	Green tea (fresh)	Green tea (dried)
Colour	Green	Greyish black
Odour	Shoothing characteristic	characteristic
Taste	Bitter astringent	Bitter astringent
Touch	Slightly rough	Rough

3.2. Microscopical characteristics

3.2.1 Leaf microscopy

Samples analyzed presented consistent characters as described in the literature for the *Camellia sinensis* species Figure: ii) shows the presence of anomocytic stomata located on the abaxial surface Figure: iii) Shows presence of unicellular and thick trichomes and figure:iv) Described as typical anatomical markers of the species as per the references and confirming the identity of the plant material

Fig 2 Front view of the

Fig 3 Unicellular Trichomes

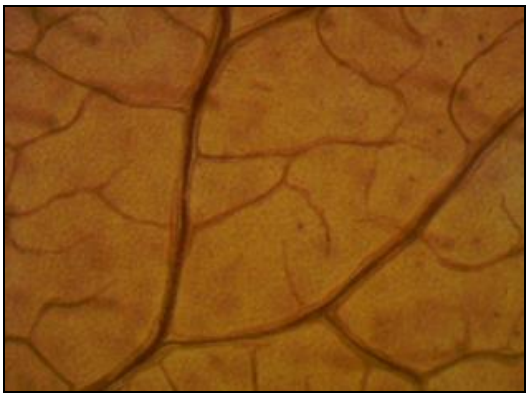

Fig 4 Typical anatomical mark fragement of leaf

3.3. Qualitative Analysis of Green Tea

Table 2: (Physicochemical evaluation)

6	Physico - Chemical Evaluation	LoD (Loss on Drying)	7.90%
		Total Ash	4.3%
		Acid Insoluble Ash	0.80%
		Alcohol Soluble Extractive	41.20%
		Water Soluble Extractive	50%
7	Assay	Total Polyphenols	23.75% (Dry mass basis)

3.4 Quantitative Analysis Results of Green Tea by Tea Research Association, Tocklai Tea Research Institute, Jorhat-08, Assam India

Table 3: (Assay)

SL. No	Parameter	% (Dry mass basis)
1	Total Polyphenol	23.1
2	Caffeine	3.81
3	Total Catechins	20.93

3.5 Chromatographic Profile of Crude Ethanolic Extract of Green Tea leaf

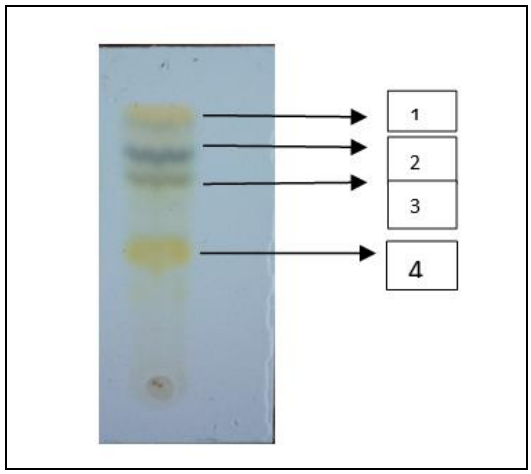

Fig 5: The result of separation green tea crude extract by TLC using silica gel GF_{254}

The details of solvent system and the Rf values are mentioned in the Table-4.

Table 4: (TLC parameters)

Extract	Solvent system	No. of spots	Rf values
Ethanol	Hexane: Diethyl ether: Acetone (5:4:2)	4	Spot-1 0.93 Spot-2 0.59 Spot-3 0.41 Spot-4 0.35

3.5 Phytochemical analysis of crude leaf aqueous extract

Table 5: (Phytochemical screening)

Phytochemicals	Petroleum ether	Acetone	Chloroform	Ethanol (70%)
Phytosterols	Present	Present	Present	Present
Carbohydrates	Present	Present	Present	Present
Saponins	Absent	Absent	Absent	Absent
Tannin	Present	Present	Present	Present
Alkaloids	Present	Present	Present	Present
Flavonoids	Present	Present	Present	Present
Anthrocyanins	Absent	Absent	Absent	Absent
Proteins	Present	Present	Present	Present
Terpenoids	Absent	Present	Absent	Absent
Glycosides	Present	Present	Present	Present

4. Discussion

Macroscopic and microscopic characters are one of the important criteria for identification. The analysis of the chemical and physical standards are the confirmatory tests for identification. The different leaf ages produce different tea qualities since their chemical compositions are different. The results of the stomata distribution showed that between stomata and distribution in the leaves are hypostomatic. Stomata associated to photosynthesis to produce food for plants. Stomata is the entrance of CO2 which is one of the raw materials in the process of photosynthesis. Photosynthesis also altering the synthesis of secondary metabolites in tea plant including catechin, and also polyphenol was correlated to the weather. It causes the relation between the photosynthesis and secondary metabolites content such as catechins. The presence of a lot of unicellular trichomes on both sides of the tea leaf creates a microenvironment of water vapor around the leaf. The result of macroscopic and

microscopic characterization of the leaves can determine the anatomical structures of plant metabolite storage. The quality of herbal medicines relies on their bioactive constituents. Therefore, TLC fingerprinting serves as an important and powerful tool for standardization and determination bioactive compounds. Green tea leaves extract represented the distinctive TLC spots that similar to the Rf values of the catechin. The composition of polyphenol as catechin reported was higher (major compound), it is indicated that astringency was mainly determined by the content of catechins and the other phenolic compounds. Green tea leaves have a simple processing without any fermentation process and higher content of polyphenols especially catechins derivatives. It has been confirmed by the determination of the polyphenol, catechins. According to the results of the phytochemical analysis in green tea leaves extract, there is the presence of alkaloid, flavonoid, tannin, and saponin. Green tea leaves extract contains the high concentration of catechins**.**

However, each type of tea has a number of different content depending on tea processing, geographic location, and growth condition of the tea plant. Polyphenols compound in green tea is a powerful antioxidant agent and positive effect on health. In earlier study also reported that green tea leaves ethanolic extract has an antidiabetic activity [15].

5. Conclusion

The current study revealed that the result of macroscopic and microscopic characterization of the leaves can determine the anatomical structures of plant metabolite storage. The present work can be used to provide data about the pharmacognostic characteristics of the green tea leaves of Camellia sinensis that proves the importance of these results. This study will be helpful in the pharmacognostic and phytochemical identification of green tea leaves extract, also the result of identification will be fingerprinted for the proper identification of crude drugs from green tea leaves and provide pharmacological preparation in the future. The higher yield of polyphenol as well as cathechin may be a potent factor for reducing the blood glucose level of diabetic patients**.** Further clinical studies can be carried-out for the scientific establishment of the beneficial effects of green tea collected from Maheswari Tea Estate**.**

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