Extraction And Isolation of Tannins from *Camellia sinensis* L. (Tea Leaf)

Thu Zar Win[1], Khaing Phyu Win[2], Thida Win[3], Moe Win[4] and Myo Thida Chit[5]

Abstract

Camellia sinensis L. of the family of Theaceae is known to be used in tea leaves that is drunk daily. The compounds containing tannin present in tea leaves were determined by phytochemical tests. Tannin was extracted from tea leaves and obtained 16 % of tannin. Tannin class was also detected. Melting point of condensed tannin was found to be 212-213 ºC. Functional groups present in tannin were determined by FT-IR spectrum. Extracted tannin was employed as a natural mordant. Many classes of tannins have antioxidant properties, which have been found to be lower total cholesterol, lower blood pressure and stimulate the immune system.

Keywords*: Camellia sinensis* L., Tannin, FT-IR , Melting point, Phytochemical

Introduction

Tea leaf is consumed by more than two thirds of the world's population and is the most popular beverage next only to water (Fessenden, R.J. and Fessenden, J.S., 1990). This drink has been consumed for thousands of years and this long safety record presents its medicinal properties and achieve chemical constituents such as tannins, caffeine, enzymes and essential oils. According to literature, tea leaves contain 15 % of tannin based on dry weight (Alschler L.,1998). Tannins are defined as naturally occurring water soluble polyphenolic compounds of high molecular weight (about 500-3000) containing phenolic hydroxyl groups to enable them to form effective cross links between protein and other macromolecules (Haslam, E.,1996).

Tannins are found in a wide variety of plant parts such as bark wood, fruits, fruit pods, leaves, roots and plant galls. They are largely used in medicine, preparation of inks, preservation of lather, food industry and in the dyeing process. Tannin are classified into two main groups; (a) hydrolysable tannins and (b) condensed tannins (Kulkarni,S.S., et. al., 2011).

Botanical Aspect of *Camellia sinensis* L.

Scientific Name	:	*Camellia sinensis* L.
Family Name	:	Theaceae
Myanmar Name	:	Laphet
English Name	:	Tea
Part used	:	Leaf
Medicinal Uses	:	Lower total cholesterol, Lower blood pressure, Stimulate the immune system

[1] Demonstrator, Department of Chemistry, University of Mandalay
[2] Demonstrator, Department of Chemistry, University of Mandalay
[3] Demonstrator, Department of Chemistry, University of Mandalay
[4] Associate Professor, Dr, Department of Chemistry, University of Mandalay
[5] Professor, Dr, Department of Chemistry, University of Mandalay

Figure 1. Nature of tea leaves

Materials and Methods

Collection of Sample

Tea leaves were collected from Taunggyi, Shan State. Firstly, the leaves were cleaned, and chopped into small pieces. These were allowed to air in the well-ventilated room for two weeks. They were made into powder by grinding machine and then stored in the container before use.

Preliminary Phytochemical Screening of Tea Leaves

Preliminary phytochemical investigation of tea leaves was carried out for various tests such as alkaloid, flavonoid, phenolic compound, reducing sugar, glycoside, polyphenol, tannin and saponin respectively. These tests were carried out on the tea leaves sample according to the report methods of Harbone. (Harbone, J.B.1984)

Extraction of Tannin from Tea Leaves

Tea leaves powder 10 g was extracted with water 120 mL at boil for 1 hour and after cooling it was filtered through a fine muslin cloth and the filtrate was collected separately. The remaining residue was extracted by three more times, in order to complete the extraction. The total extract 200 mL was heated to boil and was allowed to stand overnight and filtered again. The clear filtrate was concentrated in a water bath and treated with saturated brine solution. A brownish color precipitate thus obtained was filtered and dried in an oven. The yield percent of tannin was calculated based on raw material.

Qualitative Determination of Tannin Class from Extracted Tannin

The qualitative analysis was carried out by treating 0.5 % solution ofthe extracted tannin product with various reagents such as aqueous ferric chloride, 1 % gelatin solution, 10 % lead acetate and copper II sulfate solution. The color change after the addition of reagents were noted (Prabhu, K.H and Teli, M.D,2011).

Determination of Melting Point of Extracted Tannin from Tea Leaves

Melting point of extracted tannins was determined at the Department of Chemistry, Yadanabon University.

Determination of Functional Groups of Extracted Tannin from Tea Leaves by FT-IR Spectroscopy

The functional groups present in extracted tannin were determined by using FT-IR spectrometer [Hyper-IR/SHIMAD24), Department of Chemistry, University of Mandalay.

Results and Discussion

Preliminary phytochemical test of Tea Leaves

Preliminary phytochemical tests were done to investigate the presence or absence of chemical constituents in tea leaves.

Table 1. Results of Preliminary Phytochemical Tests of Tea Leaves

No.	Constituents	Reagent	Observation	Inference
1	Alkaloid	1 % HCl, Dragendorff's solution	Orange color	+
2	Flavonoid	EtOH, Mg turning. Conc: HCl	Pink color	+
3	Phenolic compound	EtOH, 10 % $FeCl_3$ solution	Green color	+
4	Polyphenol	EtOH, 1 % $FeCl_3$, 1 % $K_3[Fe(CN)_6]$ solution	Blue color	+
5	Reducing sugar	Distilled water, Benedict's solution	Brick red color	+
6	Tannin	EtOH, Conc : H_2SO_4, 1 % gelatin solution	Jelly like	+
7	Saponin	Distilled water	Froth	+
8	Glycoside	Distilled water, 10 % lead acetate solution	Yellow color	+

+ = Present

According to the phytochemical tests, tea leaves contain alkaloids, flavonoid, phenolic compound, polyphenol, reducing sugar, tannin, saponin and glycoside. Among these compounds, tannin plays an important role in dyeing process because it can be used as mordant to sustain coloring matter permanently in using materials.

Qualitative Determination of Tannin Class from Tea Leaves

The yield percent of extracted tannin was found to be 16 %. In order to find the class of tannins, a set of qualitative tests were carried out with various reagents and their results were shown in Table 2 and Figure 2.

Table 2. Qualitative Analysis of Tannin

No.	Reagents	Observation
1	2 % Gelatin	Dirty white precipitate
2	5 % Aqueous ferric chloride	Dark green precipitate
3	10 % Lead acetate	Pinkish precipitate
4	Copper II sulfate solution	Faint green
5	Dilute hydrochloric acid solution	Dark red solids
6	Dilute sulfuric acid solution	Flesh colored precipitate

Figure 2. Characteristic color change of tannin with various reagents

Extracted tannin produced a dark green precipitate with aqueous ferric chloride and dark red solids with dil HCl solution. It clearly confirms that tea leaf extract is condensed tannins which are vitally important in dyeing process. This tannin gives pale brown color on wool yarn.

Condensed tannins are basically flavonoid dyes and do not possess any adverse side effects and hence they are favorable for user.

Figure 3. Before and after dyeing with extracted tannins

Melting Point Determination of Extracted Tannin from Tea Leaves

In order to know how much tannin is purified, tannin was checked by melting point determination. According to the literature, pure tannin can be obtained between the melting point range of 210 – 215 ºC. In this study, the result of melting point obtained, 212 – 213 ºC lies between the accessible range of melting point of pure tannin. Therefore, the extracted tannin was found to be pure.

FT-IR Spectrophotometric Analysis

The FT-IR spectrum of extracted tannin was recorded by using perkin Elmor spectrophotometer at the Department of Chemistry, University of Mandalay. Figure 4 shows the FT-IR spectrum of tannin and the band assignments are described in Table 3.

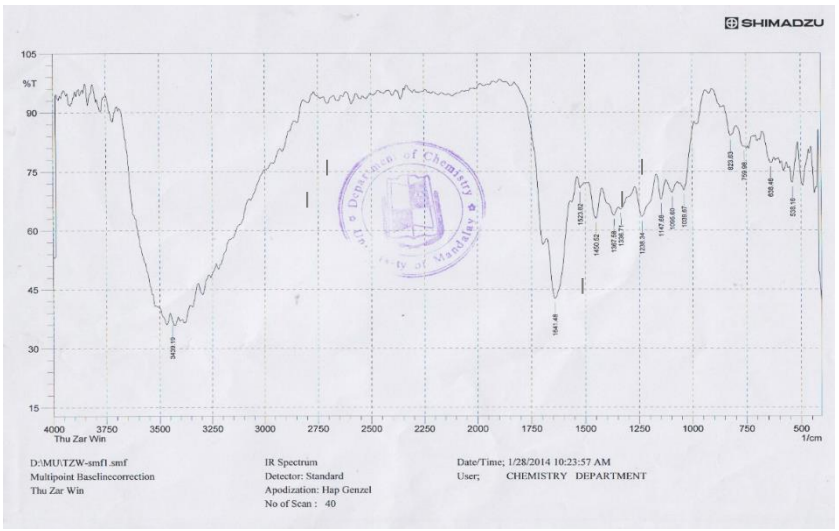

Figure 4. FT-IR Spectrum of extracted Tannin

Table 3. FT-IR Assignment of Extracted Tannin

No.	Wave number (cm^{-1})	Band Assignments
1	3439.19	– OH stretching vibration of alcohol group
2	3085.25	C–H stretching vibration of sp^2 hydrocarbon
3	2933.83	C–H stretching vibration of sp^3 hydrocarbon
4	1612.54 1523.82 1450.52	C=C ring skeletal stretching vibration of aromatic benzene ring
5	1367.58 1338.71	– OH in plane banding vibration of alcohol group
6	1238.34	C–O stretching vibration of alcohol group
7	1147.68 1095.0 1039.67	asymmetric and symmetric C–O–C stretching vibration of ether group
8	823.83 759.98	C–H out of plane bending vibration of aromatics group
9	650.46	–OH out of plane bending vibration of alcohol group

According to FT-IR spectrum, extracted tannin from tea leaves contains OH stretching vibration of alcohol group, C–H stretching vibration of sp^2 hydrocarbon, C–H stretching vibration of sp^3 hydrocarbon, C=C ring skeletal vibration of sp^3 hydrocarbon, C–O stretching vibration of alcohol group, asymmetric and symmetric C–O–C stretching vibration of ether group, C-H out of plane bending vibration of aromatics group, and OH out of plane bending vibration of alcohol group. There is a good evidence for the characterization of extracted tannin (Silverstein, and Webster,1998).

Conclusion

Chemical investigation of this medicinal plant, namely *Camellia sinensis* L. (Tea - Leaf) was selected for the present work. Phytochemical investigations of tea leaves contain various chemical constituents such as alkaloid, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, polyphenol and tannin respectively. The 16 % of tannin compound was obtained from this selected sample. Among these compounds, tannin plays an important role in dyeing process because it can be used as mordant to sustain coloring matter permanently in using materials.

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