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Physicochemical Parameters of Black Tea and Antibacterial Activity of Extracted Caffeine

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Abstract: *Percentage of total ash, water extract, water-soluble ash, acid insoluble ash, crude fiber was estimated gravimetrically. The alkalinity of water-soluble ash was determined by titration with a basic aqueous solution of NaOH. The amount of total ash, water extract, water-soluble ash, alkalinity of water-soluble ash, acid insoluble ash and crude fiber are 12.76, 48.25, 49.00, 0.80, 0.92, and 13.04%, respectively. Caffeine was also isolated from tea sample and characterized by FT-IR and UV-Vis spectrophotometry and melting point. Antibacterial activity of extracted caffeine was studied against Escherichia coli and Staphylococcus aureus bacteria. Extracted caffeine shows resistant properties against Staphylococcus aureus.*

Keywords*: Gravimetric analysis, Caffeine extraction, Spectrophotometric characterization, Antibacterial activities.*

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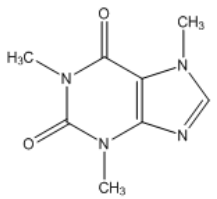
I. Introduction

Tea is the largest consuming drink in the world and cultivated in more than thirty countries around the world. In addition, it is one of the most commonly consumed caffeinated beverages in the world and its worldwide consumption is second only to water **[1-5]**. In fact, percent of moisture, total ash, water extract, lipids, water-soluble ash, acid insoluble ash, crude fibre content are some important parameter of tea samples **[1-5]**. Gravimetric analysis is widely used to estimate these parameters. Gravimetric analysis is the process of isolating and weighing an element or a definite compound of the element in as pure a form as possible **[6]**. Moreover, another characteristic parameter is alkalinity of water-soluble ash and, it is determined by titrated with base **[4]**. Black tea is a source of caffeine that stimulates the central nerves system, relaxes smooth muscle in the airway to lungs, stimulate the heart and act on the kidney as diuretic **[7, 8]**.



Caffeine

Caffeine is slightly polar organic molecule due to the presence of two carbonyl groups and four nitrogens in the structure. Caffeine is more soluble organic solvents much closer to its own polarity, e.g; dichloromethane than that in water. Thus far, isolation of caffeine and other water soluble materials of tea leaves by using hot water is a common method. The caffeine is extracted from the water after cooling with dichloromethane. Other water-soluble components of tea, tannins are slightly soluble in dichloromethane. But when sodium carbonate is added to the extract the tannins will be converted to phenolic anions. Thus phenolic salts are formed from phenolic anions, which are not soluble in dichloromethane but soluble in water.

$$\underset{\text{tannins}}{ArOH} + Na_2CO_3 \longrightarrow \underset{\text{tannins salt}}{ArONa} + NaHCO_3$$

Thus, caffeine can be isolated from tea sample using dichloromethane **[9, 10]**.

Especially, caffeine is widely used as a central nervous stimulant and reported to be an inhibitor of microorganism growth. It has antimicrobial and antioxidant activities **[11-14]**. Disk diffusion is based on the determination of inhibitor or resistance zone, which is proportional to the antibacterial activity of the antibacterial disk. **[15-16]**.

In the current context, the percent of total ash, water extract, water-soluble ash, acid insoluble ash, crude fibre and alkalinity of water-soluble ash are estimated from tea sample, which is collected from open market in Sylhet, Bangladesh. Moreover, caffeine is isolated from collected tea sample and thoroughly characterized by spectroscopic technique. The antibacterial activity of isolated caffeine is studied by disk diffusion method.

II. Material And Methods

2.1. Materials and Techniques

All the solvents (acetone, methanol, dichloromethane, n-hexane, hydrochloric acid) used were the analar grade. Nutrient agar, beef extract, peptone, sodium chloride, sodium carbonate were purchased from Sigma-Aldrich.

Infrared spectrum (IR) for the caffeine was recorded by a SHIMADZU Prestige-21 FT-IR spectrometer in the range of 400–4000 cm^{-1} at room temperature using KBr plate.

UV-Vis spectrum for the caffeine was collected on a SHIMADZU 1800 PC UV-Visible spectrophotometer in the spectral range of 200–8000 nm at room temperature.

Melting points for the caffeine was obtained with an electro-thermal GALLENKAMP melting point instrument.

Tea sample was heated on Muffle furnace JEIO-TEC for determination of ash, water extract, crude fiber content etc.

Bacteria were cultured in an incubator Binder RL-53 by using laminar airflow. In antibacterial activity test all apparatus were used after sterilizing by using an autoclave.

2.2. Methods

2.2.1. Determination of Total Ash

The black tea sample was ground to pass through the number 30 mesh sieve. 5 g of the ground sample was taken in a pre-weighed china dish and the contents were ashed to 525±25 °C for 30 min. The process was repeated until the constant weight of two consecutive weighing samples. The ash content of the sample was expressed in percent by the mass of taken sample **[4]**.

2.2.2. Determination of Water-Soluble Ash

20 mL of boiled water was added to the total ash content, and the mixture was boiled for 5 min, then cooled and filtered. The filtrate was reserved for the estimation of alkalinity of water-soluble ash. The residue and the filter paper were returned to the same crucible and the contents were ash to 525±25 ºC for 30 min, then cooled and weighed again. The process was repeated so that the two consecutive weighing samples became the same weight. From the weight of the total ash content and the weight of ash left after filtration, and the amount of water-soluble ash was calculated and expressed on a percent of the total ash **[4]**.

2.2.3. Determination of Alkalinity of Water-Soluble Ash

The filtrate obtained from the water-soluble ash estimation was titrated against 0.1N HCl using methyl orange as indicator. From the titrate value, the alkalinity of the water-soluble ash was calculated as KOH percent by mass of the taken **[4]**.

2.2.4. Determination of Acid Insoluble Ash

5 g of the ground sample was taken in a pre-weight porcelain beaker and the contents were ashed to 525±25 °C for 30 min, then cooled and 25ml of 37% HCl was added. The contents were boiled, then cooled and filtered. The residue was washed with hot water to remove the traces of acid. The filter paper with residue was returned to the same crucible and the contents were ashed again to 525±25 °C for 30 min, cooled and reweighed. The process was repeated until there was no difference in two consecutive weighings. The acid-insoluble ash content of the sample was expressed in percent of the taken sample **[4]**.

2.2.5. Determination of Water Extracts

200 mL of boiling water was added to 2 g of tea sample in a 1000 mL flask fitted with an air condenser of 1m length. The contents were boiled gently for one hour. The contents were cooled and filtered through pre-weighed sintered glass crucible, and finally washed with hot water. The contents were dried in an oven to 103±2

°C for 16 h, then cooled and weighed. From the total weight of the sample and the weight of dried residue left after extraction, the amount of water extract was calculated and expressed on a dry matter basis **[4]**.

2.2.6. Determination of Crude Fibre Content

About 2.5 g of tea sample was digested with 200 mL of hot 0.255N H_2SO_4 for 30 minutes in a1000 mL flask fitted with a condenser. The contents were filtered and transferred to 200 ml of hot 0.312N NaOH using the same set up like that for the acid digestion and filtered through sintered glass crucible. The residue was washed with hot water, 1.0% HCl, methanol and acetone, respectively. The contents were dried in an oven to 103±2 °C for 3 h, then cooled and weighed. The residue was ashed by keeping the crucible in a muffle furnace to 525±25ºC for 3 h. The dish was cooled and weighed again. From the weights of the crucible with residue and crucible with ash, the crude fiber content of black tea sample was calculated and expressed as a percent on dry matter basis **[4]**.

2.3. Extraction of Caffeine

30 mL of deionized water and 2.0 g of $CaCO_3$ were added to the 5 g tea sample in a beaker. The contents of the beaker were gently boiled for 10 min in a water bath. The hot liquid was decanted into a 50 mL Erlenmeyer flask, and then 20 mL of deionized water was added to the beaker and boiled again. Further, the liquid was decanted in the beaker. Finally, the tea extract was cooled to room temperature and separated by 5 mL of dichloromethane using a separatory funnel. A small amount of sodium sulfate was added to the organic layer to remove the water. After filtering, the filtrate was evaporated and, then get the dried yellowish-white crude caffeine. Finally, the pure white caffeine was obtained from the crude product through the recrystallization using the mixture of acetone and n-hexane. The pure caffeine was characterized using melting point and spectroscopic methods **[9]**.

2.4. Antibacterial Studies

Antibacterial activity of isolated caffeine was performed by disk diffusion method **[15, 16]**.

2.4.1. Preparation of the Culture Media

The bacteria were grown in the nutrient broth which was prepared by the mixture of beef extract (1.5 gL^{-1}), yeast extract (1.5 gL^{-1}), peptone (5 gL^{-1}) and NaCl (5 gL^{-1}) in distilled water. The prepared broth medium was heated for 15 minutes to complete dissolution and was autoclaved for another 15 min. Then, the single colony of *Escherichia coli* and *Staphylococcus aureus* bacteria were added in 20 mL broth separately and incubated at 37 °C for 24 h.

2.4.2. Preparation of Sample Disk

A stock solution of 50 mg mL^{-1} was prepared by dissolving extracted caffeine in distilled water. Paper discs of Whatman filter paper (0.45 micro-pore) of uniform diameter (5 mm) and thickness (1mm) were sterilized. 10 microliter of stock solution (500 µg sample) was socked in each disk.

2.4.3. Preparation of Agar Plates

Bacteriological nutrient agar (35 gL^{-1}) was suspended in water. The mixture was autoclaved to 120 °C for 15 min and then dispensed into sterilized Petri dishes, and finally allowed to solidify and used for inoculation.

2.4.4. Procedure of Inoculation

Inoculation was done with the help of micropipette with sterilized tips, 25 µL of activated bacterial strain was placed onto the surface of an agar plate, and spread evenly over the surface by using a sterilized bent glass rod.

2.4.5. Application of Disks

Sample disks and antibiotic disks were applied in each earlier inoculated agar plates and incubated to 37 °C for 24 h. The zone of inhibition (diameter) was then measured (mm) around the sample and standard antibiotic disks. Antibiotic ciprofloxacin (CIP) was used as a standard antibiotic disk.

III. Results and Discussion

3.1. Gravimetric and Titrimetric Analysis

Gravimetric analysis data of quantification of lipids, total ash, water-soluble ash, acid insoluble ash, water extract, and crude fiber content and titrimetric analysis data of estimation of alkalinity of water-soluble ash of collected sample are given in **Table 1**.

Table 01: Parameters of tea sample

Name of analysis	Observed value (%)
total ash	12.76
water-soluble ash	49.00
alkalinity of water-soluble ash	0.80
acid insoluble ash	0.92
water extract	48.25
crude fibre content	13.04

3.2. Isolation of Caffeine

The melting point of the isolated white color crystalline caffeine is 235 ˚C **[17]**. The percentage of the mass of isolated pure caffeine with respect to the mass of tea leaves is 0.37 %, whereas the percentage of the mass of isolated crude caffeine with respect to the mass of tea leaves is 0.87%.

3.3. Characterization of Caffeine

3.3.1. FTIR Spectrophotometry

The FTIR spectrum of isolated caffeine is given in **Figure 01**. The Characteristic peak positions for the isolated caffeine of current studies are well matched with that of reported studies **[17, 18]**. The characteristic bonds with the corresponding stretching frequency are given in **Table 02**.

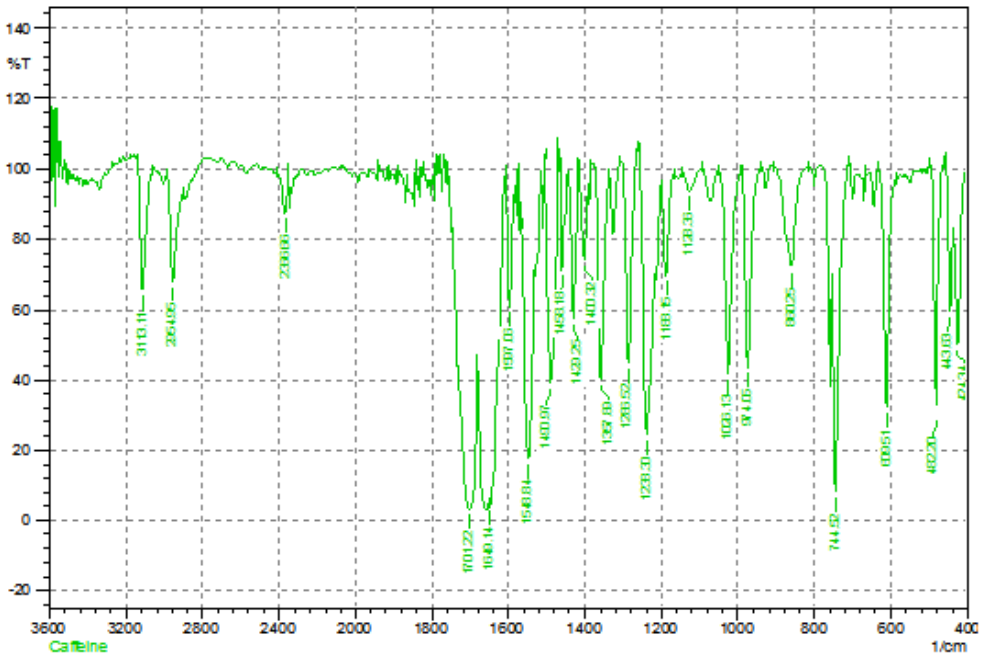


Figure 01. FTIR spectrum of isolated caffeine

Table 02: The characteristic bonds with corresponding stretching frequency are given

stretching frequency (cm^{-1})		Characteristic Bonds	Mode of vibration
Current Studies	Reported Studies **[17]**		
2955	2955	SP^3 C-H bond	Stretching
1597	1551	C=C bond	Stretching
1701	1702	C=C bond	Stretching
1238	1240	C-N bond	Stretching
1649	1661	C=N bond	Stretching

3.3.2. UV-Vis Spectrophotometry

Maximum absorption in an aqueous solution of isolated caffeine is obtained at 273 nm which is a good agreement with the standard caffeine **(Figure 02)**. Generally, purines give sharp absorption band at 270 nm, whereas, the absorption peak is shifted into 273 nm for caffeine due to the substitution of three methyl groups

on three nitrogen atoms. The characteristic absorption band at 273 nm for caffeine is attributed to the electronic transition of n–σ* and л–л*, where nonbonding electrons are available on nitrogen atoms and carbonyl groups **[9, 19]**.

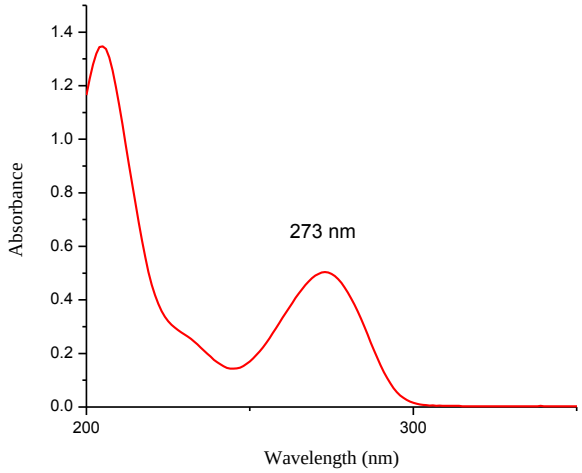


Figure 02. UV-Vis spectrum of isolated caffeine

3.4. Antibacterial Activity

Antibacterial activity of isolated caffeine was tested against *Escherichia coli and Staphylococcus aureus*, and compare with the antibacterial activity of antibiotic ciprofloxacin (CIP) against corresponding bacteria. It has been observed that caffeine shows resistant activity (create very small zone) against *Staphylococcus aureus* **(Figure 03)** and does not show activity against *Escherichia coli*, whereas antibiotic ciprofloxacin (CIP) show inhibitor activity (create large zone) against both bacteria. Antibacterial activities of caffeine against different pathogenic bacteria have been reported **[11, 12, 14]**.

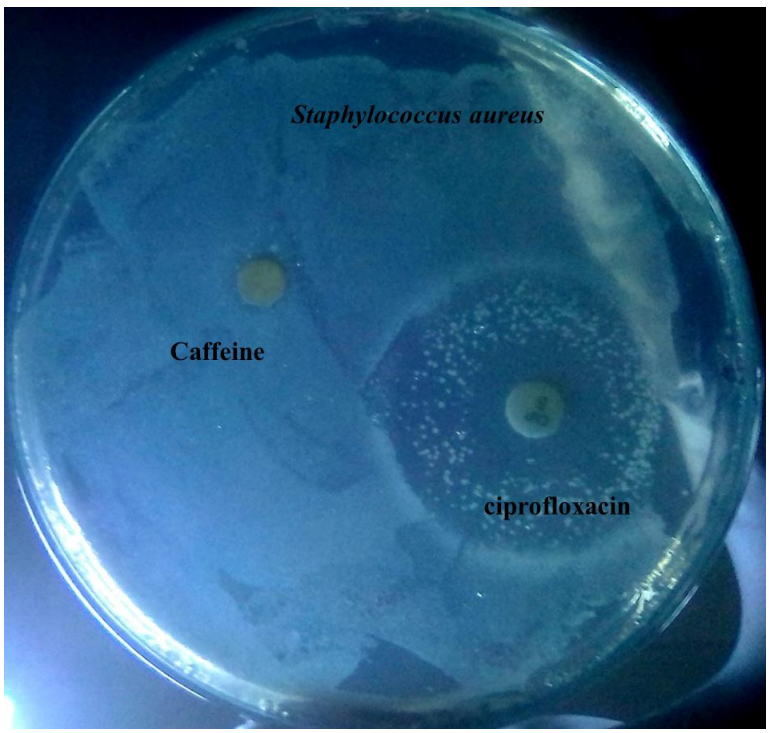


Figure 03. Antibacterial activity of isolated caffeine and ciprofloxacin against *Staphylococcus aureus* bacteria

IV. Conclusion

Total ash, water extract, water-soluble ash, acid insoluble ash, and the crude fiber content of tea in the open market of Sylhet, Bangladesh are estimated. Caffeine was isolated and characterized by FTIR and UV-vis spectroscopy, and melting points. The antibacterial activity of extracted caffeine is evaluated against *Escherichia coli and Staphylococcus aureus* by disk diffusion method.

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