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## Physicochemical and Phytochemical evaluation of different black tea brands

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### ABSTRACT

Standardization of herbal drugs raw material is essential to assess the quality, based on the consistency (concentration) of their active principles. Tea plant (*Camellia sinensis*) contains Caffeine that is responsible for the stimulating effect of the beverage. The objectives of this study are extraction, qualitative estimation of caffeine by TLC method, physicochemical standardization, phytochemical evaluation (qualitative tests) and chemical tests for detection of inorganic elements from different black tea brands. The different black tea brands (Brooke Bond Taj Mahal, Tata Premium and Duncan Double Diamond) are suddenly collected from local market. Standardization including – organoleptic properties, foreign matter, pH of aqueous solution ash values, extractive values, successive extractive values, moisture content, volatile oil content, Preliminary phytochemical screening and chemical tests for inorganic elements. The results obtained from this study can be used to standardize different black tea brands in the market.

**Key words:** Tea, *Camellia sinensis*, physicochemical standardization, phytochemical evaluation.

### INTRODUCTION

The subject of herbal drug standardization is massively wide and deep. For the purpose of research work on standardization of herbal formulations and nutraceuticals, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance (Sante et al, 1992.). The World Health Organization (WHO) has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and by applying suitable parameters and standards. In order to overcome certain inevitable shortcomings of the pharmacopoeial monograph other quality control measures must be explored (Pifferi et al, 1999. Shinde et al, 2009. Singh et al, 2004. Street et al, 2008.). Tea plant (*Camellia sinensis*) is one of the most widely consumed drinks in the world. In the Far East (Particularly in China & Japan), Tea is consumed mainly as a hot infusion of “Unfermented” fresh green shoots (Green Tea), where as in most other countries the beverage is prepared from predominantly “fermented” (Black tea) (Takami et al, 2002.). Caffeine one of the methylxanthine compound has long been known to be a natural ingredient present in coffee, tea and cola. It is highly lipophilic compound that can elevate mood; decreases fatigue, relieve tension, relax smooth muscle, bronchial muscle, stimulate CNS and Cardiac muscle and also act as a diuretic (Mukhopadhyay et al, 2003.). The common effect of caffeine when taken in high doses is increased metabolic rate, irritability, sleep disturbance and gastrointestinal aches (Ramarethinam et al, 2004.).

### MATERIAL AND METHODS

The different black tea brands (Brooke Bond Red Label, Tata Premium and Duncan Double Diamond) suddenly collected from local market. These tea brands were authenticated by Botanist of Department of Botany, B.U., Jhansi. Chemicals of Analysed reagent (AR) grade

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(LOBA Chemicals Ltd.) and standard caffeine (CDH Laboratories).

### Extraction of caffeine

Weighed accurately 10 g of black tea of each brand and placed in a 400 ml beaker. Added 50 ml of freshly distilled water, boiled and stirred slowly for 5 minutes. Cooled the beaker at room temperature in a water bath (a 1000 ml beaker with cold water in it works well for this). When cooled, decant the solution into a 500 ml separating funnel. Extracted the aqueous solution with three successive 10 ml portions of chloroform, In extracted chloroform added a small amount of Anh. Na<sub>2</sub>SO<sub>4</sub> (drying agent) and stirred the solution gently. Extracted chloroform appeared a bit cloudy and kept for 10 minutes. Poured solution leaving behind the Na<sub>2</sub>SO<sub>4</sub> on dry weighed watch glass and kept this aside to evaporate. The residue left on watch glass found as the crude caffeine and weighed. Recrystallization of the crude caffeine was done by using small volume of hot water (Paul et al, 2000.).

### Qualitative estimation of caffeine by TLC

Sample Preparation—1% Caffeine solution in chloroform .Sample Application—By micro pipette or capillary tube. Solvent system—Ethylacetate: Methanol: Water (100: 13.5: 10). Development Tank—TLC plate is placed in a development chamber at an angle of 45°. The bottom of the chamber is covered upto nearly 1mm. by the solvent. Drying—Air drying the TLC plates for 20 min. and later in oven for 3-5 min. & Plate to be cooled before spraying. Detection—First spray with Alcoholic Iodine solution and after 2 min. with Alcoholic Hcl. Chocolate brown colour observed. If spot disappears after with Alcoholic Hcl. then again spray with Alcoholic iodine (Chatwal et al,2004.Iyengar et al,1994.). Record Rf value by this formula -

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

### Rf values of different Tea Brands

Sample No.	Distance traveled by solvent (cm.)	Distance traveled by test sample (cm.)	Distance traveled by standard (cm.)	Rf of Test sample (cm.)	Rf of standard (cm.)
S-1	8.9	6.1	6.1	0.68	0.68
S-2	9.2	5.7	5.6	0.61	0.60
S-3	8.9	5.6	5.8	0.62	0.65

S-1=Brooke Bond Taj Mahal, S-2=Tata Premium, S-3= Duncan Double Diamond

### Evaluation of the Chromatogram

Qualitative Evaluation of TLC Chromatograms are produced with the aim of identify and purity of the material you obtained. For purposes of identification, it is necessary to compare

the crude caffeine to a Reference sample of commercial caffeine. i.e. to relate the Rf values of investigated substance and those of reference substance. If the Rf values agree, it is probable, but not certain, that the two spots correspond to the same substance (Mukherjee et al, 2002.).

### Physicochemical Standardization of different tea brands covering following parameters (Kokate et al, 1999.WHO,2000.Khandelwal et al, 2002.Remington,1995.)

**Organoleptic properties – Colour – Black, Odour-Characteristic, Taste-Bitter Astringent.**

**Foreign matter – Take 100 gm. of each drug sample.**

Values in percentage (%)		
Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
0.53	0.51	0.50

**pH of 1% w/v of aqueous solution -**

Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
4.90-5.45	4.93-5.46	4.96-5.48

**Ash values - Take 3 gm. of each drug sample.**

Sr. No.	Determinants	Values in percentage (%)		
		Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
1.	Total Ash	8.60	8.66	8.55
2.	Acid Insoluble Ash	1.33	1.36	1.31
3.	Water Soluble Ash	5.23	5.28	5.22

**Extractive values (cold Maceration) – Take 5 gm. of each drug sample.**

Sr. No.	Extractive Solvent	Values in percentage (%)		
		Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
1.	Distilled water	23.04	23.06	23.03
2.	Ethanol	12.64	12.67	12.62

**Successive Extractive values – Take 10 gm. of each drug sample.**

Sr. No.	Extractive Solvent	Values in percentage (%)		
		Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
1.	Petroleum ether (b.p.60-80°C)	3.67	3.69	3.66
2.	Chloroform	0.077	0.078	0.073
3.	Acetone	0.045	0.050	0.045
4.	Methanol	0.84	0.86	0.85
5.	Ethanol	0.054	0.055	0.052
6.	Distilled water	2.59	2.61	2.55

**Moisture Content (LOD%)- Take 5 gm. of each drug sample**

Values in percentage (%)		
Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
3.63	3.76	3.70

**Volatile Oil Content** – Take 20 gm. of each drug sample.

Values in percentage (%)		
Brooke Bond Taj Mahal	Tata Premium	Duncan Double Diamond
0.012	0.010	0.011

**Phytochemical evaluation (qualitative tests) for detection of Phytoconstituents from Aqueous extract of black Tea** (Ansari et al, 2005-2006.).

S. No.	Phytoconstituents	Aqueous Extract
1.	Acidic Compounds	+
2.	Alkaloids	+
3.	Carbohydrates (Polysaccharides)	+
4.	Flavonoids	+
5.	Glycosides (Anthraquinones)	+
6.	Phenolic Compounds & tannins	+
7.	Resins	-
8.	Saponins	+
9.	Sterols & Triterpenoids	+
10.	Protein and Amino acids	+

+ = Present , - = Absent

### Chemical Tests for detection of Inorganic elements

(Khandelwal et al, 2002.).

Prepare ash of drug material. Add 50% v/v HCl or 50% v/v HNO<sub>3</sub> to ash. Keep for 1 hour or longer. Filter with filtrate perform the following tests:

#### Test for Calcium

To 10 ml. Filtrate add 1 drop dil. NH<sub>4</sub>OH and saturated ammonium oxalate solution. White ppt. of calcium oxalate forms.

#### Test for Magnesium

Filter and separate while calcium oxalate ppt. Obtained above heat and cool the filtrate. Add 10% disodium hydrogen phosphate. White ppt. Of ammonium magnesium phosphate observed.

#### Test for Sodium

Flame test: Prepare thick paste of ash of drug with conc. HCl. Take paste on platinum wire loop. Introduce in bunsen flame. Golden yellow flame is observed.

#### Test for Potassium

To 2-3 ml. Test solution add few drops sodium cobalt nitric solution. Yellow ppt. Of potassium cobalt nitrite observed.

#### Test for Iron

To 5 ml. Test solution add few drops 2% potassium ferrocyanide. Dark blue coloration is observed.

## RESULTS AND DISCUSSION

Extraction and recrystallization of caffeine from different tea brands follow same procedure for each and every tea brand. Qualitative estimation of caffeine is determined by TLC method. By this method we determine the R<sub>f</sub> value of each brand of crude caffeine and compare with standard caffeine. On the basis of R<sub>f</sub> value we investigate the identity and purity of the crude caffeine. So we can say that Brooke Bond Taj Mahal has caffeine in purified form than other brands.

Physicochemical standardization is the most prominent means for quality assurance of herbal products. The foreign matter was found minimum-0.05% in Duncan Double Diamond as compare to other brands. The pH of 1% w/v of aqueous solution was minimum(4.90-5.45) in Brooke Bond Taj Mahal revealed that slightly acidic. Total Ash is important for the evaluation of purity and quality of drugs. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Total ash value content was 8.55%, Acid insoluble ash value was 1.31%, Water soluble ash value was 5.22% in Duncan Double Diamond which were minimum as compare to other. Extractive values are indicative of the presence of the polar or nonpolar extractable compounds in a plant drug. The water soluble extractive value (23.06%) and alcohol soluble extractive value (12.67%) were maximum in Tata Premium. Successive extractive values were maximum in Tata Premium. Lowering of these extractive values indicate the addition of exhausted material with the original drug.

Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high or Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principle. Moisture content (LOD) was found minimum in Brooke bond Taj mahal (3.63%).

Volatile oil content was found maximum in Brooke Bond Taj mahal (0.012%). Phytochemical evaluation revealed the presence of acidic compounds, alkaloids, flavanoids, anthraquinone glycosides, phenolic compounds and tannins, sterols and triterpenoids, saponins, carbohydrates (polysaccharides), proteins and amino acids and absence of resins. Chemical tests indicate the presence of inorganic elements- Ca, Mg, Na, K and Fe.

## CONCLUSION

The results obtained from physicochemical and phytochemical evaluation of different black tea brands can be used for the standardization.

WHO parameters discussed here, can be considered as the identifying parameters to authenticate the drug.

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