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**International Journal Of Pharma Professional's
 Research**
Research Article



**PHYSICOCHEMICAL AND PHYTOCHEMICAL
 EVALUATION OF DIFFERENT MARKETED BRANDS
 OF BLACK TEA**

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ISSN NO:0976-6723

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Abstract

The objectives of present study were extraction, physicochemical standardization, phytochemical evaluation (qualitative tests) and chemical tests for detection of inorganic elements in different black tea brands. Brands selected for the study Anand Special, Marvel Rojana, Marvel Glory had foreign matter (0.50%, 0.53%, 0.51%) respectively. Water soluble extractive values were 25%, 28%, 22% in Anand Special, Marvel Rojana, Marvel Glory respectively. Moisture contents were 0.5%, 5.2%, 1.3% in Anand special, Marvel Rojana, Marvel Glory respectively. Inorganic contents were found as printed on label. On phytochemical screening it was found that all three brands were positive for the presence of carbohydrate, protein, amino acids, alkaloids and flavonoids.

Keywords: - : Anand special, Marvel Rojana, Marvel Glory, Caffeine

Introduction

Tea is one of the most popular and lowest cost beverages in the world and consumed by a large number of people. Owing to its increasing demand, tea is considered to be one of the major components of world beverage market. The global market for hot beverages (coffee and tea) is forecasted to reach US\$69.77 billion in value and 10.57 million tons in volume terms by the year 2015. [1]

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 & Cardiac muscle and also act as a diuretic. [2]

Coffea arabica and *Coffea robusta* are the two important classes of coffee belong to the family rubiaceae. Coffee has been used traditionally in the treatment of asthma, atropine-poisoning, fever, headache, jaundice, malaria, migraine, narcosis, sores and vertigo. Coffee enemas have been used for cancer. Caffeine is the most important constituent of coffee which is widely used as stimulant. A number of beneficial health properties have been attributed to coffee; among them are diuretic, antimicrobial and antioxidant activities.

Constituents such as such as caffiene, chlorgenic acid, caffiec acids condensed pro anthocyanidins, quinic acid, and ferulic acid have been reported to anti-oxidant and possess anti- bacterial activity. [3]

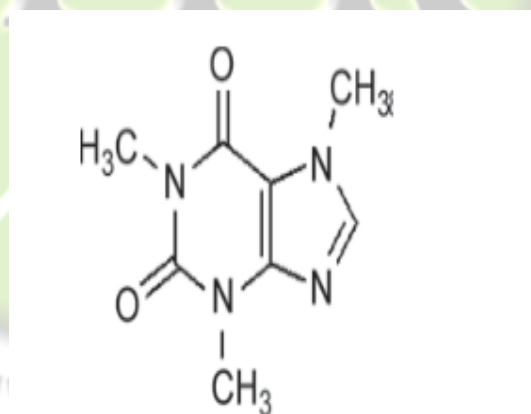


Figure 1: Chemical formula of caffeine⁴

Material And Methods:-

The different black tea brands (Anand special Batch No- AT-01 Packaging Date Feb-2014, Marvel Rojana Batch No- R/786-51 Packaging Date Mar-2014 and Marvel Glory Batch No-786/GL-17 Packaging Date- March 2012) collected from local market of Moradabad U. P. India.

Extraction of Caffeine[5,6]

Weighed accurately 80 g of black tea of each brand and placed in a 500 ml soxhlet Colum. Added 500 ml of freshly distilled water, boiled for complete soxhletion process. The aqueous extracted was filtered while hot. Extract was treated with lead acetate to precipitate tannins and filtered; excess of lead acetate present in the solution is precipitate with dilute H₂SO₄ in the form of lead sulphate. Filtered solution was boiled with charcoal to remove collaring matter and filtered to remove charcoal. Filtered decolorized solution is extracted with CHCl₃ by separating funnel with three times separation and chloroform was evaporated, after evaporation caffeine were obtained as white material and recrystallized with alcohol.

Qualitative Estimation Of Caffeine By TLC[7,8]

1% Caffeine solution was prepared in chloroform & this solution was applied by capillary tube on TLC plate. Solvent system -Ethyl acetate: Methanol: Water (100: 13.5: 10). Development tank-TLC pate is placed in a development chamber at an angle of 45°. The bottom of the chamber is covered up to 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 color observed. If spot disappears after with Alcoholic HCl. then again spray with Alcoholic iodine. Record R_f value by this formula –

. Solvent system—Ethyl acetate: Methanol: Water (100: 13.5: 10). Development Tank—TLC plate is placed in a development chamber at an angle of 45°.

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

Determination of physicochemical parameters:**Determination Of Moisture[9]****Procedure:**

- Weight about 5 g of the powered drug into a weight flat and thin porcelain dish.
- Dry in the oven at 100 OC.
- Cool in a desiccators and watch. The loss in weight is usually record as moisture.

Determination Of Foreign Matter: [10]**Procedure:**

- Weight 100 g of the sample
- Spread the sample on a white tile or a glass plate uniformly without overlapping.
- Inspect the sample with necked eyes.
- Separate the foreign matter .
- After complete separation, weigh the matter and determine % w/w present in the sample.

Determination of Ash Values of A Crude Drug**Total Ash Value****Procedure:**

Heat a silica crucible to red heat of 30 min, allow to cool in desiccators and weight unless otherwise specified in the individual monograph. Weight accurately about 3 g of the substance being examined and evenly distribute it in the crucible. Dry at 100 OC to 105 OC for 1 h and ignite to constant weight in a muffle furnace at 600 OC ± 25 OC, allow the crucible to cool in a desiccator after each ignition. Calculate the percentage of ash value [11].

Acid Insoluble Ash Value**Procedure:**

Boil the ash with 25 ml of 2M hydrochloric acid for 5 min; collect the insoluble matter on ash less filter paper. Wash ash with hot water ignite, cool in a desiccators dry and weight. Calculate the percentage of acid insoluble ash with reference to air dry [11]

Water Soluble Ash

To the crucible containing the total ash add 25 ml of water and boil for 5 min, collect the insoluble matter on ash less filter paper, wash with water and ignite for 15 min at a temperature not exceeding 450 OC. Weight of this residue in mg from the weight of total ash. Calculate the content of driedwater soluble ash in mg/g [11].

Determination Of Extractive Values:[10]**Alcohol soluble extractive value****Procedure:**

- Weight about 5 g of the drug in a weighing bottle and transfer it to a dry 250 ml. conical flask
- Fill a 100 ml graduated flask to the delivery mark with the solvent (90%alcohol). Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.
- Cork the flask and set aside for 24 hours, shaking frequently. (maceration)
- Filter into a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml. of the filtrate to a weighed , thin porcelain dish, as used for the ash value determinations.

V. Evaporate to dryness on a water bath and complete the drying in an oven at 100°C.

VI. Cool in desiccator and weigh.

VII. Calculate the percentage w/w of extractive with reference to the air dried drug.

Water soluble extractive value

Procedure:

I. Weigh about 5 g of the drug in a weighing bottle and transfer it to a dry 250 ml. conical flask

II. Fill a 100 ml graduated flask to the delivery mark with the water and add few drops of chloroform. Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.

III. Cork the flask and set aside for 24 hours, shaking frequently. (maceration)

IV. Filter in to a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml. of the filtrate to a weighed, thin porcelain dish, as used for the ash value determinations.

V. Evaporate to dryness on a water bath and complete the drying in an oven at 100°C.

VI. Cool in desiccator and weigh.

VII. Calculate the percentage w/w of extractive with reference to the air dried drug.

Phytochemical screening of caffeine:

❖ Tests for carbohydrate (polysaccharides):

• Iodine test: Mix 3 ml. test solution and few drops of dilute iodine solution. Blue color appears; it disappears on boiling and reappears on cooling [10].

• Tannic acid test: with 20% tannic acid, test solution gives ppt [10].

❖ Test for Proteins and amino acids:

• Biuret test: To 3 ml. test solution add 4% NaOH and few drops of 1% CuSO₄ solution. Violet or pink color appears [10].

• Ninhydrin test: Heat 3 ml. test solution and 3 drops 5% Ninhydrin solution in boiling in water bath for 10 min. Purple color or bluish color appears [10].

❖ Test for alkaloid:

• Murexide test: Caffeine was taken in a petridish to which hydrochloric acid and potassium chlorate are added and heated to dryness. A purple color was obtained by exposing the residue to vapors of dilute ammonia. The purple color was lost on addition of fixed alkali [11].

❖ Test for Tannins:

• Lead acetate solution was added in test solution white precipitate were not appeared [10].

• Bromine water was added in the test solution the bromine water not decolorized [10].

• Dilute HNO₃ was added in test solution reddish to yellow color not appeared [10].

❖ Test for flavonoids:

• Shinoda test: To dry powder or extract, add 5 ml. 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings pink color observed [10].

• To small quantity of residue, add lead acetate solution, yellow colored precipitate was formed [10].

❖ Test for Vitamin C:

• To 2 ml of a 2% w/v solution add 2 ml of water, 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate, shake and allow standing; a deep violet color is produced. Add 5 ml of 1 M sulphuric acid, the color disappear [10].

Chemical Tests for detection of Inorganic elements^[10].

Prepare ash of drug material. Add 50% v/v HCl to ash. Keep for 1 hour. Filter & with filtrate perform the following tests:

Test for Calcium

To 10 ml filtrate adds 1 drop dil. NH₄OH and saturated ammonium oxalate solution. White ppt. of calcium oxalate forms. ppt is soluble in HCl but insoluble in acetic acid.

Test for Magnesium

Filter and separate while calcium oxalate ppt. obtained above i.e. in calcium test. Heat and cool the filtrate which with solution of sodium phosphate in dilute ammonia gives white crystalline precipitate.

Test for Sodium

Flame test: Prepare thick paste of ash with conc. HCl. Took paste on platinum wire loop & introduce in bunsen flame. Golden yellow flame is observed.

Test for Potassium

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

Test for Iron

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

Result & Discussion

Physicochemical Standardization of different tea brands covering following parameters

Organoleptic properties –

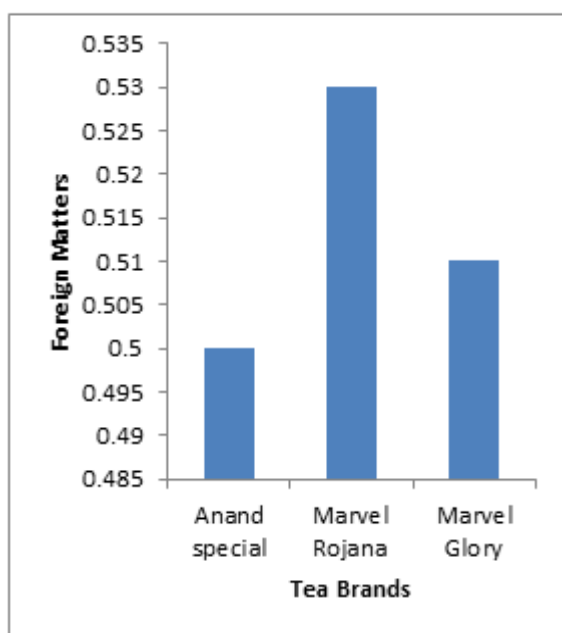
Colour – Black,

Odour- Characteristic,

Taste- Bitter Astringent.

Table No-1 Foreign matter: 100 gm. of each drug sample was taken.

Values in percentage (%)		
Anand special	Marvel Rojana	Marvel Glory
0.50	0.53	0.51



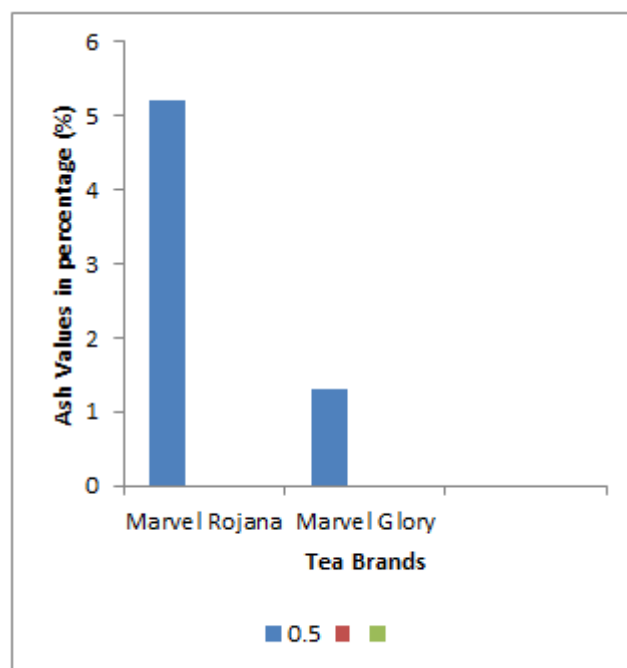
Graph No-1. Comparison of foreign matter

Table No:-2 pH of 1% w/v of aqueous solution –

Anand special	Marvel Rojana	Marvel Glory
5.0-5.40	5.30-5.90	5.80-6.00

Table No:-3 Ash values: 3 gm. of each drug sample was taken.

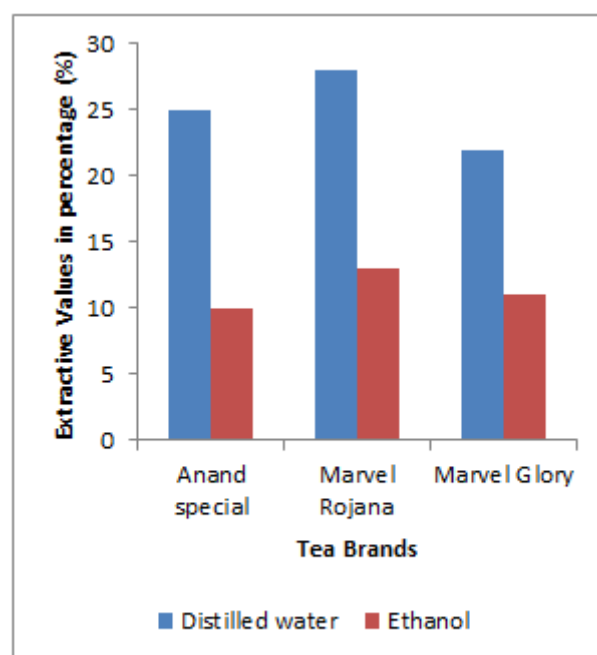
S No	Determinants	Values in percentage (%)		
		Anand special	Marvel Rojana	Marvel Glory
1	Total Ash	23.34	16.67	23.34
2	Acid Insoluble Ash	16.07	9.03	16.10
3	Water Soluble Ash	20.23	13.29	20.01



Graph No-2 Comparison of ash value

Table No:-4 Extractive values (cold Maceration): 5 gm. of each drug sample was taken.

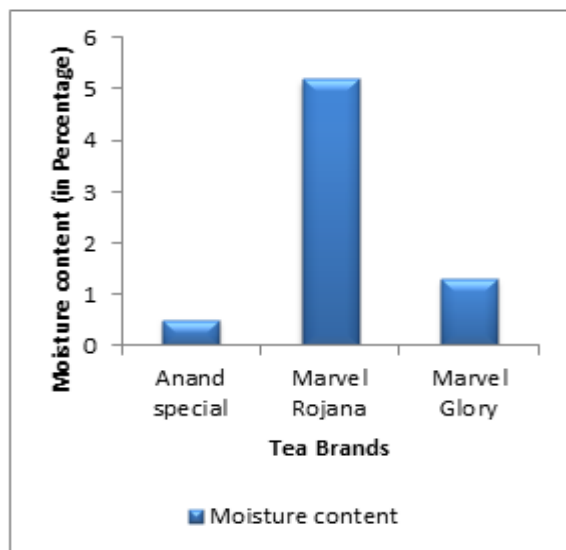
S No	Determinants	Values in percentage (%)		
		Anand special	Marvel Rojana	Marvel Glory
1	Distilled water	25	28	22
2	Ethanol	10	13	11



Graph No-3 Comparison of extractive values

Table No:-5 Moisture Content (LOD%)- 5 gm. of each drug sample was taken.

Values in percentage (%)		
Anand special	Marvel Rojana	Marvel Glory
0.5	5.2	1.3

**Graph No- 4 Comparison of moisture contents****Table No:-6 R_f values of different Tea Brands**

Sample	Distance traveled by solvent (cm.)	Distance traveled by test sample (cm.)	Distance traveled by standard (cm.)	R _f of Test sample (cm.)	R _f of standard (cm.)
Anand special	8.6	5.9	5.8	0.68	0.67
Marvel Rojana	9.1	6.3	5.9	0.67	0.64
Marvel Glory	8.4	5.3	5.1	0.63	0.60

Table No:-7 Chemical Tests for detection of Inorganic elements

Sr. No	Chemical constituents	Aqueous Extract		
		Anand special	Marvel Rojana	Marvel Glory
1	Test for Calcium	+	+	+
2	Test for Magnesium	+	+	+
3	Test for Sodium	+	+	+
4	Test for Potassium	-	+	+
5	Test for Iron	+	+	+

+ = Present, - = Absent

Table No:-8 PHYTO CHEMICAL SCREENING

S. No	Chemical constituents	Aqueous Extract		
		Anand special	Marvel Rojana	Marvel Glory
1	Carbohydrate (Polysaccharides)	+	+	+
2	Proteins and amino acid	+	+	+
3	Alkaloids	+	+	+
4	Tannins	-	-	-
5	Flavonoids	+	+	+
6	Vitamin C	-	-	-

+ = Present, - = Absent

For qualitative estimation of caffeine, the R_f value of each brand of crude caffeine was compared with standard caffeine. On the basis of R_f values identity and purity of the crude caffeine was determined. It was found that Anand special had caffeine in more purified form than other brands. The foreign matter was found minimum-0.50% in Anand special as compared to other brands. The pH of 1% w/v of aqueous solution was minimum (5.0-5.40) in Anand special when compared to other brands. 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 found to be 16.67%, Acid insoluble ash value 9.03%, Water soluble ash value 13.29 % respectively in Marvel Rojana which were minimum as compared to other brands. The water soluble extractive value (28%) and alcohol soluble extractive value (13%) were found to be maximum in Marvel Rojana. Deterioration time of the plant material depends upon the amount of water present in plant material. High water content 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 Anand special (0.5%). Phytochemical evaluation revealed the presence of alkaloids, flavanoids, carbohydrates (polysaccharides), proteins and amino acids and absence of tanins and vitamin C. Chemical tests indicated the presence of inorganic elements- Ca, Mg, Na, K and Fe.

CONCLUSION:

The consumption of energy drinks with high caffeine content has increased markedly in recent years, now a day's many brands are available in market with content of many effective and harmful constituents which has affected the human health. Thus, the present study demonstrates the quality of deferent marketed brand of tea. The results obtained from physicochemical and phytochemical evaluation of different marketed brands of black tea may be used for the standardization. The parameters like ash values, extractive values, moisture content etc were evaluated in the study. These parameters are demonstrating the quality of marketed brand. It may be concluded that all the three black tea brands fulfill the requirements printed on lable.

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