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# DEVELOPMENT OF CITRUS BASED BEVERAGE UTILIZING BACOPA MONNIERI

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**ABSTRACT** The study was done to formulate a drink from an old medicinal herb and retain all the potential benefits with a new taste and flavor. For this an herbal drink was formulated and its quality ascertained. In the first part of the study, syrup was prepared from the raw leaves of the herb with addition of acids and flavors. Then this syrup was diluted further followed by carbonation with 1:3 ratio of soda water and bottled. Three samples were prepared namely, T1 (same as previous but with 1:3 ratio carbonation and dividing the sample hot filled and cold filled ). In the next part, prepared samples were subjected to sensory evaluation,chemical and microbial analysis when fresh and after regular intervals at room temperature (27±1 °C) and refrigerated temperature (below $7^0$C). Microbial analysis of the product was done to check the quality of the herbal drink and self-life of the product. The control sample T1 cold filled was the most acceptable due to its unique taste and flavor, followed by sample T1( hot filled) . The present study entailed to conclude that preparation of a drink with *B. monnieri* leaf extracts gives a new taste and flavor with high nutritional values. This drink can be stored safe for nearly a month if carbonated and storage at refrigerated temperature (below $5^0$C).

**KEYWORDS :**

## INTRODUCTION:

***Bacopa monnieri*** (**waterhyssop**, **herb of grace**, **Indian pennywort**) is a perennial, creeping herb native to the wetlands of southern and Eastern India, Australia, Europe, Africa, Asia, and North and South America. *B. monnieri* is an herb used in Ayurveda.

Bacopa is commonly used for Alzheimer's disease, improving memory, anxiety, and attention deficit-hyperactivity disorder (ADHD) among many other uses. But there is limited scientific research to support these uses.

Phenolic compounds are ubiquitous in plant organs. They are secondary metabolites consisting of an aromatic ring with different degrees of hydroxylation. Phenolics are derived from biosynthetic precursors such as pyruvate, acetate, aromatic amino acids such as phenylalanine and tyrosine, acetyl CoA and malonyl CoA following the pentose phosphate, shikimate, and phenylpropanoid metabolism pathways. Phenolic compounds occurring in herbal beverages include phenolic acids, coumarins, flavonoids, tannins,lignans and lignins.

Antioxidants are known for their ability to inhibit or delay the oxidation of other molecules in food and biological systems. They are protective against oxidative stress via different mechanisms and modes of action that are often independent of their antioxidant effect and may render their effects cooperatively via several mechanisms. These modes of action include freeradical scavenging, singlet oxygen quenching, inactivation of peroxides and other ROS, metal ion chelation, quenching of secondary oxidation products, and inhibition of pro-oxidative enzymes, among others.

Consumption of sugar-sweetened beverages may be one of the dietary causes of metabolic disorders, such as obesity. Therefore, substituting sugar with low calorie sweeteners may be an efficacious weight management strategy. So steviol glycoside was used to reduce the calorific intake. Sucralose,sweet-tasting calorie free compound that may also be used as a sugar substitute or as an alternative to artificial sweeteners.

Sucralose is an artificial sweetener and sugar substitute. The majority of ingested sucralose is not broken down by the body, so it is noncaloric.Sucralose is about 320 to 1,000 times sweeter than sucrose.

## 1. MATERIALS AND METHOD:

Present study was conducted in 2018 at Techno India (Main) Salt lake, Kolkata. Fresh herbs were procured from local market while sucralose was purchased from a local pharmacy. Other raw material like sucrose was also procured from the local market and the fresh beverage was used for all the estimation.

### 1.1. Sugar:

Water is added to a cooking pot and heated.Cane sugar is added carefully and the solution is continuously stirred.

The gas oven is switched off and the brix of solution is checked. The final brix of that solution is $42^0$ brix.

### 1.2. Spice extract:

Cardamom and cinnamon are powdered using grinder and mortar and pastel.The ground spices are added at a ratio of 8:2 (Cardamom: Cinnamon).1g spice powder is added to 10 ml water.This solution is heated at $70^0$ C for 15 minutes in a water bath.The solution is filtered with muslin cloth.

### 1.3. Citrus juice:

Juice is extracted from sweet lime and lemon and juice is blended in 1:1 and 1:2 ratio.

### 1.4. Sucralose syrup:

Water is added to a cooking pot and heated.Water is added to a cooking pot and heated.The brix is made upto $11^0$ brix.

### 1.5. Herb extract:

The leaves are separated from their stems and placed in a tray for drying at $54^0$C for 30 minutes.The dried leaves are added to a beaker at a ratio of 1:10 (herb:water).The sample is heated in a water bath at $54^0$C for 1 hour.Filtered the sample with muslin cloth.

**The samples were mixed in this sequence:**

1. Sugar syrup
2. Spice extract
3. Herb extract
4. Juice mixture

The blended solution is split into 2 samples T1 hot filled and T1 cold filled.

Heated the hot filled at $95^0$C for 7 minutes and blended it with soda water at 1:3 ratio.

Cold filled sample is cooled at $7^0$C for 30 minutes and blended it with soda water at 1:3 ratio.

### 2. Quality analysis:

**Quality control tests have been followed in the following procedures:**

**2.1. pH:**
The pH of the sample is measured with pH paper and the readings are based on the colour indicated by the samples during the test.

**2.2. Total soluble solids:**
The samples are taken in a glass screen of the brix refractometer and lid covering the glass is closed and the brix content is measured using the lens provided.

**2.3. Titrable acidity:**
The burette is filled with 50 ml of 0.1 N NaOH solution.The pipette is used to take out 10 ml of the formulation and added to a conical flask.The flask is also added with 1-2 drops of phenolphthalein.

Titration is done till the sample in the flask attains a pale pink colour and the reading are noted down.

**2.4. Polyphenol content:**
Standard gallic acid should be prepared in (1:1) methanol water.Mother stock concentration is made as 500mg/ml.Standards are prepared by adding 0.75,2.5 and 3.75ml.Added 1 ml of folin ciocaltu reagent (1:5 with water)Added 3ml of 20% sodium carbonate solution and properly mixed the solutions.Allowed it to stand for 30 minutes and took the observation at a wavelength of 760nm in a spectrophotometer.1ml of both the formulations are taken.Added 1 ml of folin ciocaltu reagent into the solution.

Allowed it to stand for 30 minutes and took the observation at 760nm in a spectrophotometer.

**2.5. Total plate count:**
Prepared the media for plating in a conical flask.Cotton is plugged on mouth of conical flask and covered with brown paper. Sterilized the medium into autoclave at 121$^0$C (15psi) for 15 mins.Sterile distilled water was taken in five sterile test tubes. Then serial dilution was carried out in laminar flow in sterile test tubes. Test tube with $10^{-6}$ dilution is used for probing.2 ml sample is added first to the petriplate and then 15 ml of media is added to it. Petriplate incubated at 32$^0$C for 24 hrs. and noted down the colonies.

**2.6. Mold count:**
Prepared the czapak dox media for plating in a conical flask.Cotton is plugged on mouth of conical flask and covered with brown paper. Sterilized the medium into autoclave at 121$^0$C (15psi) for 15 mins.Sterile distilled water was taken in five sterile test tubes. Then serial dilution was carried out in laminar flow in sterile test tubes. Test tube with $10^{-6}$ dilution is used for probing.2 ml sample is added first to the petriplate and then 15 ml of media is added to it. Petriplate incubated at 32$^0$C for 24 hrs. and noted down the colonies.

**3. Results:**

**3. 1. pH:**
i)pH of hot filled sample= 4, ii)pH of cold filled sample= 4

**3.2. Total soluble solids:**

| Sample Type | Total soluble solids ($^0$Brix) |
|---|---|
| Hot filled | 6 |
| Cold filled | 3.5 |

**3.3. Titrable acidity:**

| Sample type | Sample taken (ml) | Initial burettereading (ml) | Final burette reading (ml) |
|---|---|---|---|
| Hot filled | 10 | 0 | 3.1 |
| Cold filled | 10 | 3.1 | 4.4 |

Since 1ml of 0.1 N NaOH= 7mg of citric acid
Hence,

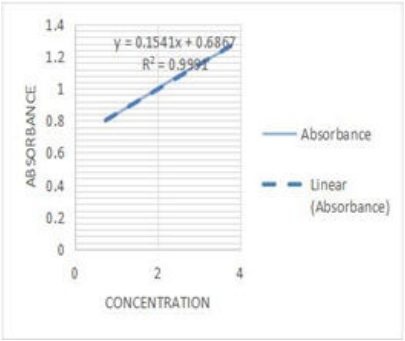
Sample 1 (Hot filled) contains=21.7 mg of citric acid/10ml of sample

Sample 2 (Cold filled) contains=30.8 mg of citric acid/10 ml of sample

**3.4. Polyphenol content:**

| Sample(mg/ml)/ Sample type | Absorbance |
|---|---|
| 0.75 | 0.799 |
| 2.5 | 1.08 |
| 3.75 | 1.26 |
| Hot filled | 0.224 |
| Cold filled | 0.379 |

**The graph that was obtained from the readings is given below:**




**The concentration of gallic acid equivalent was found out to be:**
I. Sample 1(hot filled)=89.9 mg/ml gallic acid equivalent
II. Sample 2(cold filled)=93.61 mg/ml gallic acid equivalent

**3.5. Total plate count:**
I. CFU in hot filled sample=1,000,000 CFU/ml
II. CFU in cold filled sample=0 CFU/ml

**3.6. Mold count:**
I. CFU in hot filled sample=5,500,000 CFU/ml
II. CFU in cold filled sample=3,000,000 CFU/ml

**CONCLUSION:**
After all the trials and quality analysis conducted, we come to a conclusion that cold filled sample from trial 1 is the desired product as:

It is more visually acceptable. It has better taste.

It is more protected from microbes as all the essential compounds are intact.

**For the future work, the following can be considered:**
Sucralose should be replaced by stevia as it is less harmful and many companies have used it in their carbonated beverages.Honey should not be added into the syrup

Acid regulators must be added instead of natural fruits as chances of spoilage might increase.

Spices should be removed from the sample as they don't impart significant organoleptic appeal.

Cola flavour based herbal beverage can be tried out as masses are accustomed with the taste and smell.

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