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A study on development and quality analysis of a beverage incorporated with *Terminalia chebula*

Research Article

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Abstract

The study's aim was to develop a beverage incorporated with *Terminalia chebula Retz.,* locally known as *Haritaki,* having gastro-intestinal motility improving properties. The objectives of the study were to develop the beverage, check its acceptability by conducting sensory evaluation and chemically analyzing the beverage for quality assessment by testing its anti-oxidant properties and presence of gallic acid, i.e., a hydrolysable tannin present in *Haritaki* fruit which majorly possess the properties of improving gastro-intestinal motility. The beverage was made by two methods *viz.* overnight refrigeration and boiling method. Then sensory evaluation was conducted by 9-point hedonic scale and as a result of which sample made with overnight refrigeration method were found to be more acceptable. Then samples prepared with both the methods were subjected to antioxidant property test and High-performance liquid chromatography (HPLC) to check the presence of gallic acid. The test results were in favor of the sample prepared by overnight refrigeration method having 14.49% more antioxidant property and 26.06mg/kg more gallic acid than that of the sample prepared with boiling method. The original taste of *Haritaki* is very bitter and strong. So, with the development of this beverage the flavor of *Haritaki* is enhanced and bitterness is reduced. Therefore, consumers can opt for this beverage over the original powdered form, hence increasing the acceptability of *Haritaki*. Also, no further preparation is required, people can consume it at an ease. The beverage developed will be a boon to the food industry in this era of unhealthy lifestyle.

Key Words: *Haritaki,* Hydrolysable tannin, Gallic acid, HPLC, Antioxidant property, Gastrointestinal motility.

Introduction

From earlier times medicinal plants are used to combat diseases by the humans.

These medicinal plants have been considered invaluable source of exceptional phytoconstituents.

And these constituents are extensively used in production of drugs against various ailments (4).

The traditional healthcare system of India has Ayurveda in it since forever. An ancient herb called *Terminalia chebula Retz.* also known as *Haritaki* in Hindi is called the "King of Medicine" and is always listed first in Ayurveda because of its exceptional therapeutic benefits (1,5).

Numerous common names of *Terminalia chebula Retz.* include "black myrobalan", "chebulic myrobalan", "ink tree", "*Haritaki"* (in Sanskrit and Bengali), "*Harad"* (in Hindi), "*Harada"* (in Gujarati and Marathi). Given that it cures all illnesses and is revered by God Shiva (*Hara*). It is often known as "*Haritaki*" (4).

Terminalia chebula Retz. holds anti-bacterial, anti-ulcerogenic, anti-fungal, anti-oxidant and gastric motility improving properties. In addition, *Haritaki* has played a significant role in creation of number of Ayurvedic formulations.

The fruit of *Terminalia chebula Retz.* contains plethora of functionalized bio-substances. The fruit of *T. chebula* contains significant amounts of gallic acid, total phenols and flavonoids, coumaric acid, catechin, and chlorogenic acid. Major properties which affect the gastrointestinal issues come from gallic acid and chebulinic acid (6).

The fruit possesses mild laxative, tonic, alterative, and antispasmodic qualities according to ethnobotanical usage. It helps with haemorrhoids, bleeding gums, dental caries, and oral ulcers. By mixing with water and preparing a mush, it can have analgesic, wound-healing and anti-inflammation qualities. The benefits of this substance also include appetite stimulation, gastrointestinal prokinetics, liver stimulation, stomachic, and mild laxative effects. Chronic diarrhoea has been treated with *T. chebula fruit* powder. (2).

Commercially *Haritaki* is accessible in the form of *vati* (tablet) and *churna* (powder). These forms are intensely bitter to taste which makes them slightly

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unacceptable to the consumers. Hence, the objective of the study was to develop a beverage having the properties of *Haritaki* and added natural flavors. This beverage will prove to be valuable to the food and beverage industry. The purpose of the study was to improve the acceptability of *Haritaki*.

In a study about the assessment of the aqueous extract of *Terminalia chebula Retz.* for anti-diarhheal properties, it was seen that it did not cause mortality up to a dose of 4000mg/kg (7), therefore it can be stated that the dose can safely be set below this concentration.

Materials and Methods

Preparation of the beverage

1. Preparation of *Haritaki* powder

200 g *Haritaki* fruit was procured from the local market of Faridabad, Haryana, India. 100 g of it was then roasted and pounded. The seeds were separated and the fruit was ground and kept in an air tight container.

2. Preparation of mint infused water

To prepare mint infused water, 200 g of fresh mint leaves were boiled in 1 liter of water until an aroma and change in the color of water was observed and the volume became 75% of the initial volume. The decoction was finally strained.

3. Incorporation of *Haritaki* powder in mint infused water

Haritaki powder was then incorporated in mint infused water to mask its bitterness. To make a 100ml beverage, three different concentrations of *Haritaki* powder were incorporated in 100ml mint infused water by two methods, Overnight Refrigeration (ORT) and Boiling (BT). In overnight refrigeration (ORT) technique, 0.2g/100ml (@0.2%), 0.3g/100ml (@0.3%) and 0.4g/100ml (@0.4%) of *Haritaki* powder was mixed in mint infused water and was kept for overnight refrigeration and strained after about 24 hours.

And in boiling (BT) technique, same concentrations i.e., @0.2%, @0.3%and @0.4% beverage samples were prepared by boiling (using mint infused water) and then cooling at room temperature. 1 tablespoon honey was mixed in all the beverage samples to mask their bitterness.

The beverage samples prepared by both the methods were finally set for sensory evaluation conduction.

4. Sensory evaluation

The sensory evaluation was conducted using 9-Point Hedonic Scale of 0 to 9. 0 being lowest i.e., disliked extremely and 9 being highest i.e., liked extremely. The sensory evaluation was conducted in the Food Analysis Lab of Department of Nutrition & Dietetics, Manav Rachna International Institute of Research and Studies, Faridabad. The evaluation was done by 50 consumer panelists. The samples were blinded to avoid panelist bias. Also, water bottles and crackers were provided to each panelist for de-saturating their taste buds.

The attributes evaluated were:

- Appearance
- Aroma
- Taste
- Overall acceptability.

The best results were demonstrated by the beverage samples prepared by the OR technique.

In conjunction with the literature and sensory evaluation test results, 0.4% concentration was lastly chemically analyzed for its quality characteristics.

5. Quality analysis:

The tests were conducted in the Food Testing Laboratory of National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sonepat, Haryana. Chemical analyses conducted were to check the anti-oxidant and gastric motility improving properties of the beverage.

Two samples that were subjected to testing were- Sample $A_{(BT)}$ i.e., the beverage prepared with boiling method (BT) and Sample $B_{(ORT)}$ i.e., the beverage prepared with the overnight refrigeration (ORT) method and both having the same concentrations i.e., 0.4g in 100ml (@0.4%).

The tests conducted were:

a) Antioxidant property test

The antioxidant property test was conducted by the method known as DPPH assay (2,2-diphenylpicylhydrazyl). In plant biochemistry DPPH assay is extensively used to assess free radical scavenging properties of plant constituents. As a result, DPPH method is broadly used to check the antioxidant property of plant constituents.

In this test preparation of a methanolic dilution of DPPH $1x10^{-4}$ M was done. Then four aliquots of 1ml sample in four different concentrations of methanolic extract each were prepared.

All the mixtures were kept in a dark room for 30minutes approximately. Then the absorbance of the mixtures was observed at 530nm in a UV-30 spectrophotometer. To make the blank, methanolic dilution of DPPH was used (3).

The final results of the assay were depicted in the form of percent DPPH inhibition.

b) HPLC

High performance liquid chromatography was conducted to determine the presence of gallic acid. Gallic acid is a hydrolysable tannin present in *haritaki.* This constituent of *haritaki* is majorly responsible for its gastrointestinal motility improving properties.

The chromatography was performed on HPLC-DAD (High Performance Liquid Chromatography with Photo diode array detector) instrument. Standard Gallic acid and other analytical grade reagents like Milli-Q water and acetic acid were used for the analysis.

Mobile phase was prepared by 1.8822 N-hexane sulphonic sodium salt in 1000ml Measuring cylinder and 5ml acetic acid mixed properly to the makeup volume of 1000ml with Milli-Q water.

The intermediate standard (100ppm) was prepared with 1.0ml of gallic acid in 10ml volumetric flask. The Six standard dilutions were used to create a calibration curve, calibration standard was set to a range of 1ppm-100ppm by using the mobile phase. Then the samples were diluted with 10 ml distilled water. A total of three samples were tested.

1±0.1g sample was weighed in 50ml centrifuge tube. Then 10ml mobile phase was added and mixed. Centrifuge was run at 5000rpm for 5 minutes and supernatant was taken and filtered using PVDF syringe filter and injected in HPLC for determination. The limit of quantification (LOQ) was assessed for quantitative purposes using the formula:

LOQ = 10 x σ/Slope, where σ = standard deviation of the calibration curve and slope = slope of the calibration curve.

The results obtained were that the retention time of the standard was 3.267 min, boiled sample was 3.273 min and for overnight refrigerated sample was 3.273 min too. LOQ was found to be 5 µg/ml. (Manish Pal Singh, et al., 2019).

As validated by HPLC studies, gallic acid presence was very prominent in both the samples. The results of the test were depicted in mg/kg.

Observation and Results

The results obtained from the chemical analyses were as follows:

Table no.1: Depiction of antioxidant test results

Test	Method	Sample	Result
Anti-oxidant test	DPPH	$A_{(BT)}$	38.41%
		B $_{(ORT)}$	52.9%

*Sample $A_{(BT)}$- Beverage sample (@0.4%) prepared with boiling technique; Sample B $_{(ORT)}$- Beverage sample (@0.4%) prepared with overnight refrigeration technique.

Table no.2: Depiction of Gallic acid test results

Test	Method	Sample	Result
Presence of gallic acid	HPLC	$A_{(BT)}$	59.91mg/kg
		B $_{(ORT)}$	85.97mg/kg

*Sample $A_{(BT)}$- Beverage sample (@0.4%) prepared with boiling technique; Sample B $_{(ORT)}$- Beverage sample (@0.4%) prepared with overnight refrigeration technique. The instrument used was HPLC-DAD.

Discussion

The precise dosage set in the beverage was 0.4 g *Haritaki* powder incorporated in 100ml mint infused water. Hence, the drink can be reliably taken 2-3 times a day according to the extent of the ailment.

The samples were tested in triplicate to substantiate the results of both samples A(BT)and B(ORT).

The results of the tests conducted were as follows:

Antioxidant test (through DPPH Assay) showcased sample A(BT) to be possessing lesser antioxidant property (38.41%) than sample B(ORT) (52.9%).

By HPLC, the presence of gallic acid was measured which was seen as 59.91mg/kg in sample A and 85.97mg/kg in sample B.

Both the results concluded that the antioxidant property and gallic acid were estimated more in the sample prepared with overnight refrigeration (OR) technique than the sample prepared with boiling (B) technique. That inferred that the sample made with ORT possessed much more functional benefits as compared to the sample prepared with BT. This could be due to the fact that BT hampered some of the naturally occurring components of harad, whereas the ORT thereby made no change since there was no change in the temperature.

Conclusion

The results indicated that the *Haritaki* beverage developed delivered the functions originally found in *Terminalia chebula Retz.* fruit having an edge by bearing less bitterness and no requirement of preparation. The chemical analyses concluded that the beverage had both antioxidant properties and gallic acid, which acts majorly upon gastrointestinal issues.

The ingredients in the beverage other than *Haritaki* powder and mint leaves were fennel seeds and honey. The ingredients not only had a role in masking the bitterness but also have their own functional roles such as having anti-oxidant properties.

This beverage can improve gastrointestinal motility related issues and can be taken in gastrointestinal tract diseases such as ulcer, constipation, flatulence and diarrhoea.

Human trials can be conducted under medical surveillance to check the effect of the beverage on the ailments to take the study further.

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