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ORIGINAL ARTICLE

Herbal tea with bacoside loaded saponins: formulation and characterization for food fortification from *Bacopa monnieri* L

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Abstract In recent times, there is a renewed interest of herbal tea because of growing consumer awareness for the health benefits of herbal tea consumption in the covid era. *Bacopa monnieri* is a natural medicine mainly used to enhance nerve impulse transmission, improves memory and mental function as well as the enhancement of other cognitive function because of the presence of triterpenoid saponins and bacoside A. The present experiment was conducted for the development of herbal tea with known health benefits from *Bacopa monnieri* using different spices or herbal ingredients combinations, *i.e*., Pepper, cardamom, and ginger to increase its aesthetic properties. A higher sensory score for pepper-brahmi was found superior with 8.71 (colour), 8.43 (aroma), 8.43 (taste), 8.29 (after taste), and 8.46 (overall acceptability) over three combinations and control. Jal brahmi tisane revealed, 0.036 ± 0.0004 mg/100 ml Bacoside A, 87.72 mg TE/100 ml TAC, 106.02 mg GAE/100 ml TPC, 21,100 µg/100 ml calcium, and 87 µg/100 ml iron, which is essentially good for human health.

Keywords *Bacopa monnieri* · Bacoside A · Tisane · Antioxidants

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Abbreviations

TPC	Total phenolic content
TAC	Total antioxidant capacity
TE	Trolox equivalents
GAE	Gallic acid equivalents
mg	Milligram
ml	Milliliter
µg	Microgram

Introduction

Herbal medicine is regularly used by 80% of the world population and is increasing in popularity day by day. The world is putting more effort to improve the health of the population and their living standards due to increasing poverty and population. Natural products from plants, animals, and other sources are the base of the treatment for many human diseases. Medicinal and aromatic plants are an essential part of natural wealth and it has a special place in the sociocultural, spiritual, and healthcare arena. Herbs are known to be harmless medicines having no or minimal side effects; therefore, people consume them without hesitation. Nowadays people are becoming more health-conscious in terms of the prevention of diseases as well as their treatment with the help of herbal medications, resulting in the increased use of plant-based products including herbal tea (Viljoen and Vermaak 2012).

Bacopa monnieri (also known as Jal brahmi) (Sanskrit), brahmi (Kannada), sambranichettu (Telugu), thyme-leafed gratiola, water hyssop, herb of grace (Silpa et al. 2019). It is a creeping perennial herb with small oblong leaves and purple to whitish flowers, found in warm wetlands, and native to Australia and India. Jal Brahmi is commonly used as a memory enhancer in the ayurvedic medicinal system since the ancient era that found as a weed in the rice field. It can grow naturally in wetlands, shallow water, damp and muddy shores (Thorat et al. 2018). The entire plant is used for various medicinal purposes. It is known to be used for memory enhancement, improving learning concentration, and relieving anxiety. The brain tonic property is mainly because of the presence of saponins, especially Bacosides. This plant is considered one of the 'celestial drugs' when consumed with milk for six months (Saran et al. 2021). This miracle herb has the inherent potential of enhancing memory and vitality, therefore, gaining attention for its commercial cultivation globally (Saran and Patel 2019). The antioxidant-rich Jal Brahmi increases immunity against pathogens and bacterial infection. When Jal brahmi is consumed as a tea or chewed, it enhances respiratory health by clearing out phlegm and mucus.

Tisanes are an infusions, popularly known as herbal tea. Tisane or herbal tea is a non-caffeine-containing beverage prepared through an infusion of any part of any plant other than *Camellia sinensis* (true tea). They are prepared from a mixture of dried herbs, grasses, nuts, barks, seeds, fruits, flowers, or other botanical materials which provide health benefits and acceptable taste. Tisanes are an effective and inexpensive way to get the health benefits of herbs and spices. It is an important source of antioxidants, mainly phenolic components, which can prevent disease manifestation by reducing free radical-induced damage in the biological system (Piljac-Zegarac et al. 2013). Tisane provides hydration to the body without caffeine, supports heart health, reduces stomach and digestive problems, nourishes the nervous system, and strengthens the immune system (Table 1). It stimulates internal organs, avoids cold and cough, and also detoxifies the body. As it contains antioxidants in good amounts, it helps in stress relief and anti-aging (Killedar and Pawar 2017). Tea is generally consumed for its attractive aroma and taste. As some herbs and medicinal plants gives bitter and astringent taste; other spices and herbal ingredients such as ginger, mint, pepper, and lemon are added to increase their aesthetic properties and better taste (Keating et al. 2015). Considering the great importance and potential of bacosides and antioxidants derived from *Bacopa monnieri*, in human health, the study is conducted for the development and formulation of *Bacopa monnieri* tisane.

Materials and methods

The study was conducted at the College of Food Processing Technology and Bio-Energy and Polytechnic in Food Science and Home Economics, Anand Agricultural University, Anand during 2020–21 for the preparation of Jal brahmi Tisane combined with different ingredients.

Raw material

Bacopa monnieri (L.) Pennell germplasm (INGR21238) was used in the present experiment for Tisane preparation. For the preparation of Brahmi tisane, fresh aerial parts of Brahmi (leaf and stem) were selected and collected from ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand with other ingredients such as ginger, pepper and cardamom which were procured from the local market. Brahmi leaves with stems were washed with running tap water. Undesirable parts like dried leaves and rotten parts were removed and cut into uniform lengths of 3–4 cm. Prepared Brahmi was stored in HDPE bag at refrigerated temperature.

Development and formulation of brahmi Tisane

Optimized ultrasonic parameters and drying temperature were used for the formulation. Sensory evaluation was conducted to find the degree of acceptability of the herbal tisane. For making Brahmi tisane, filter paper dip bags were used. Dried leaves were made into small pieces by hand and filled into dip bags. Tea was prepared by boiling water to

Table 1 Independent and dependent variables in experiment

Treatment combinations/Ingredients	Independent Variables	Levels	Dependent variables	Constant parameters
Ultrasonication	Amplitude (%)	3	Bacoside A (%) TAC (mg trolox/g) Drying time (Min)	On time and off time
	Treatment time	3		
Drying	Temperature	3		Air velocity
Optimization of weight of Dried Leaves per Dip bag	Weight of dried leaves	4	Sensory characteristics	Water volume, temperature (°C), dipping time
Optimization of dipping time	Time	4	Sensory characteristics	Weight of dried leaves, Water volume, temperature (°C)
Development of brahmi tisane with ginger	Amount of ginger	3	Sensory characteristics	Dipping time, water volume, temperature
Development of brahmi tisane with cardamom	Amount of cardamom	3	Sensory characteristics	Dipping time, water volume, temperature
Development of brahmi tisane with pepper	Amount of pepper	3	Sensory characteristics	Dipping time, water volume, temperature

90–95 °C and infusing in a glass containing 100 ml of hot water. Based on preliminary trials, the weight of ingredients was finalized. The nine-point hedonic scale was used and attributes such as colour, aroma, taste, and after taste was scored by a panel consisting of 7 trained members.

Standardization of weight of dried leaves per dip bag

The approximate weight of dried leaves was considered by preliminary studies. For the standardization, 0.5, 1.0, 1.5, and 2 g of dried leaves were taken for making tisane. Filled bags were dipped in a glass containing 100 ml of hot water for 2 min and based on sensory attributes, the weight per dip bag was standardized. Commercially available Lipton green tea was used as a control.

Standardization of dipping time

The standardized weight of dried leaves was kept in dip bags and dipped in 100 ml of hot water for different dipping times of 1, 2, 3, and 4 min. Based on sensory attributes, dipping time was standardized. Commercially available Lipton green tea was used as a control.

Development of brahmi tisane with different spices

Brahmi tisane was formulated with different spices such as ginger, pepper, and cardamom. Weight of dried leaves and dipping time were considered from the previous standardization.

Combination with ginger

Coarsely ground, the dried ginger powder was mixed with a standardized weight of dried leaves for the development of ginger incorporated Brahmi tisane. Tisane was prepared with various proportions of ginger with Brahmi and evaluated for sensory attributes. Brahmi tisane without any spices was used as control.

Combination with cardamom

Coarsely ground, dried cardamom powder was mixed with a standardized weight of dried Brahmi for the preparation of cardamom incorporated Brahmi tisane. Tisane was prepared with the various proportions of cardamom with Brahmi and control was evaluated for sensory attributes.

Combination with black pepper

Black pepper powder was mixed with a standardized weight of dried Brahmi for the development of pepper-incorporated Brahmi tisane. Tisane was prepared with various proportions of pepper with Brahmi and control was evaluated for sensory attributes.

Phytochemical evaluation of developed brahmi tisane

Estimation of total antioxidant capacity (TAC)

The total Antioxidant Capacity (TAC) of Brahmi was determined using the procedure described by Benzie and Stain (1996). This procedure is based on the conversion of ferric form in Ferric Tripyridal Triazine complex to ferrous form and colour changes in the presence of antioxidants. About 100 mg of dried sample was taken and mixed with 5 ml of 80% methanol. It was kept in a shaker at 30 rpm for 30 min. Centrifugation at 3000 rpm for 10 min gave clear supernatant. It was made up to known volume with 80% methanol. From that solution, 20 µl was taken in a test tube and the volume made up to 300 µl with distilled water. 1.8 ml of freshly prepared FRAP reagent was added and incubated for 10 min at 37 °C. The coloured complex was measured at 593 nm using a UV spectrophotometer. For blank 300 µl, distilled water was used. Standard series of known concentrations of Trolox and volume made up to 300 µl with distilled water. All the samples and standards were treated in the same way. A Trolox standard curve was used to calculate TAC and was expressed as the equivalent weight of mg of Trolox equivalent per 100 g of sample.

$$\text{TAC(Trolox equivalent)} = \frac{\text{Standard concentration} \times \text{OD of sample} \times \text{Total volume made up} \times 100}{\text{OD of standard} \times \text{Aliquot taken} \times \text{Weight of sample}}$$

Estimation of total phenolic content (TPC)

The Total Phenolic Content (TPC) of Brahmi was evaluated using Folin–Ciocalteau method as explained by Singleton et al. (1999). About 100 mg of dried sample was taken and mixed with 5 ml of 80% methanol. It was kept in a shaker at 30 rpm for 30 min. Centrifugation at 3000 rpm for 10 min gave clear supernatant. It was made up to a known volume with 80% methanol and 0.1 ml of extract was taken in a test tube and 1 mL of Folin-Cioclateau reagent (1: 10: F–C reagent: distilled water) was added to the test tube. After 3–4 min, 2.5 ml of 20% sodium carbonate

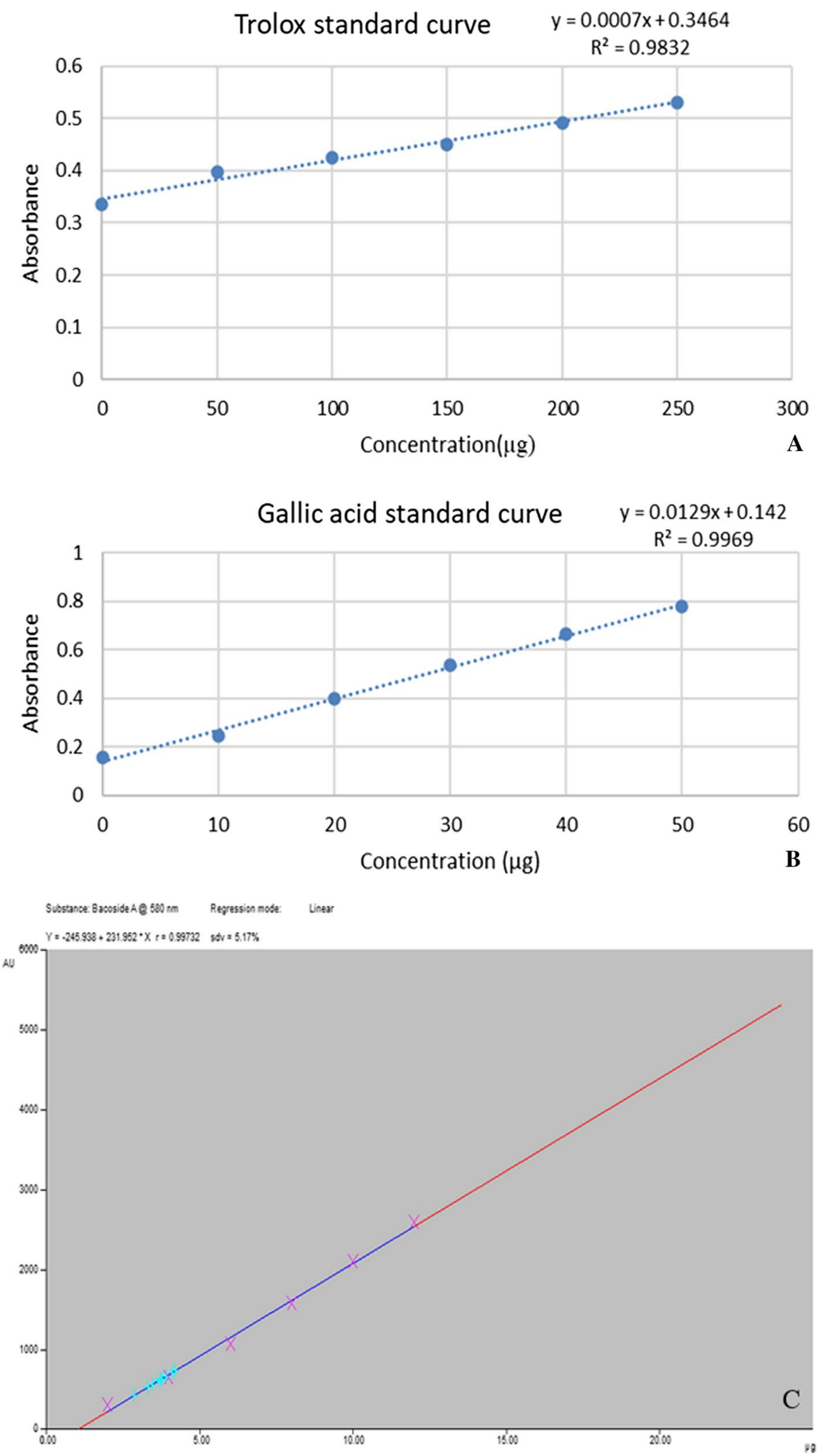

Fig. 1 Gallic acid, Trolox and Bacoside A standared curve

was added and the mixture was kept for incubation for 30 min in dark. The absorbance of blue coloured solution was taken at 765 nm using a UV spectrophotometer. Gallic acid was used as standard and was treated the same as the sample. Gallic acid standard curve (Fig. 1) was used and the concentration of TPC was expressed as mg of Gallic

Acid Equivalent /100 g of sample and calculated by using the following formula:

$$\text{TPC(Gallic Acid equivalent)} = \frac{\text{Standard concentration} \times \text{OD of sample} \times \text{Total volume made up} \times 100}{\text{OD of standard} \times \text{Aliquot taken} \times \text{Weight of sample}}$$

Estimation of bacoside A

HPTLC method was used for the estimation of Bacoside A content (Saran et al. 2022). Reference standard Bacoside A (95.23% purity) was purchased from PhytoLab GmbH & Co. A stock solution was prepared in methanol (1 mg/ml). Pre-coated TLC silica gel 60 F254 plates (E. Merck) was used as the stationary phase and ethyl acetate: methanol: water in the ratio 7.5:1.5:1 was used as a mobile phase. CAMAG twin trough development chamber was used and saturated with mobile phase for 15 min. A 20% trichloroacetic acid in chloroform was used as a derivatising reagent. The developed plate was examined under CAMAG TLC visualizer at 254 nm, 366 nm, and 540 nm wavelengths. CAMAG Scanner 3 was used for scanning.

For the analysis, dried and powdered samples were kept in methanol (1 mg/ml). After 24 h supernatant was separated out by centrifugation and solvent was evaporated off. Dried materials were again dissolved in methanol and used for HPTLC quantification. Five microliters of sample and different concentrations (2, 4, 6, 8, 10, and 12 µl) of standards were applied on a TLC plate (20 × 10 cm) with 6 mm sharp bands by using Camag Linomat V applicator. The plate was dried and kept in the saturated chamber with a 20 ml mobile phase. Development distance of 8 cm was marked and the plate was developed for that distance (12–13 min). The TLC plate was removed and dried at 105˚C. The plate was derivatised by dipping in derivatising reagent ($FeCl_3$). The derivatised and dried plate was scanned under 540 nm. The concentration of Bacoside A in the sample was calculated by comparing it with the areas measured for the standard and sample solutions. The percentage of Bacoside A in the sample was calculated by using the following formula:

$$\text{Bacoside A(\%)} = \frac{\text{Concentraion} \times 100 \times 10 \times \text{Volume made up} \times 100}{\text{Purity} \times \text{Standard weight} \times \text{Aliquot taken} \times \text{Sample Weight}}$$

Estimation of calcium and iron

About 1 g of powdered sample was digested with 30 ml of the di-acid mixture (nitric acid: perchloric acid: 2:1). The solution was heated at 180–200 °C until white fumes evolved. The cooled solution was filtered and made up to a known volume with distilled water. The mineral content of the solution was analyzed in ICP-OES (Inductively Coupled Plasma- Optical emission Spectroscopy).

Result and discussion

Development and formulation of brahmi tisane

Standardization of weight of dried brahmi and dipping time per dip bag

A study was carried out to optimize the weight of Brahmi per dip bag. Lipton green tea bag contains 1.3 g in each dip bag; however, Brahmi is different from *Camelia sinensis*. Different levels such as 0.5, 1.0, 1.5, and 2.0 g were prepared by dipping in 100 ml of hot water. Sensory evaluation scores showed that the tisane with 1.5 g of dried Brahmi showed the highest scores for aroma, taste, after taste, and overall acceptability. The highest sensory scores except for colour were obtained for the tisane with 1.5 g dried Brahmi. The scores were 7.29 ± 0.29 (colour), 7.43 ± 0.20 (aroma), 7.43 ± 0.30 (taste) and 7.71 ± 0.29 (after taste). Overall acceptability was found 7.46 ± 0.16 (Table 2).

Four levels of dipping time were decided and sensory evaluation was carried out. Different dipping times such as 1, 2, 3, and 4 min were analyzed by dipping 1.5 g of dried Brahmi in 100 ml hot water. Sensory evaluation showed that dipping times of 1 and 2 min were less liked by the panellists because of the light aroma, and colour. By dipping for 4 min, scores for aroma, taste, and after taste were less due to its strong taste and flavor. The tisane made by dipping for 3 min showed the highest acceptability score, and is statistically significant (Table 3). Dipping time of 3 min was optimized with the highest sensory scores for all the attributes *i.e.* of 7.86 ± 0.26 (colour), 8.00 ± 0.31 (aroma), 8.571 ± 0.20 (taste), 8.14 ± 0.26 (after taste) and overall acceptability 8.14 ± 0.20. Statistical analysis showed that there was a significant difference between optimized dipping time and control. However, the scores of Brahmi tisane with a dipping time of 3 min was having more sensory scores than control, Therefore, Brahmi tisane was accepted as per optimized sensory scores and parameters (Table 2).

Table 2 Effect of dipping time and amount of Jal brahmi on sensory attributes of Brahmi tisane

Dipping time	Colour		Aroma		Taste		After taste		Overall acceptability	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Control	8.00[a]	0.22	7.71[a]	0.29	7.57[b]	0.20	7.43[a]	0.20	7.68[b]	0.09
1 min	6.14[b]	0.34	5.57[d]	0.20	5.86[d]	0.26	5.86[b]	0.26	5.86[c]	0.14
2 min	7.71[a]	0.29	6.86[c]	0.14	7.00[b]	0.22	7.57[a]	0.30	7.29[b]	0.17
3 min	7.86[a]	0.26	8.00[a]	0.31	8.57[a]	0.20	8.14[a]	0.26	8.14[a]	0.20
4 min	8.14[a]	0.26	7.71[a]	0.29	6.14[c]	0.26	5.71[b]	0.18	6.93[b]	0.19
C.D	0.80		0.73		0.67		0.71		0.43	
SE(m)	0.28		0.25		0.23		0.25		0.15	
SE(d)	0.39		0.36		0.33		0.35		0.21	
C.V	9.65		9.33		8.67		9.32		5.44	
Weight of jal brahmi										
Control	7.57[a]	0.37	7.29[a]	0.29	7.29[a]	0.18	7.29[a]	0.18	7.36[a]	0.16
0.5 g	6.00[a]	0.31	5.43[b]	0.20	5.14[c]	0.26	5.71[b]	0.29	5.57[c]	0.13
1 g	6.71[a]	0.29	6.57[a]	0.20	6.57[b]	0.30	6.43[a]	0.20	6.57[b]	0.11
1.5 g	7.29[a]	0.29	7.43[a]	0.20	7.43[a]	0.30	7.71[a]	0.29	7.46[a]	0.16
2 g	7.71[a]	0.29	6.86[a]	0.26	6.29[b]	0.18	6.71[a]	0.18	6.89[b]	0.12
C.D	0.90		0.68		0.73		0.68		0.40	
SE(m)	0.31		0.23		0.25		0.23		0.14	
SE(d)	0.44		0.33		0.35		0.33		0.19	
C.V	11.57		9.19		10.12		9.16		5.36	

Values in columns with different superscripts with small letters (a and b) differ significantly ($p < 0.05$)

Formulation of brahmi tisane with different combinations:

Four levels of the weight of ginger were chosen and sensory evaluation was carried out in this study. Previously optimized, 1.5 g of dried Brahmi was mixed with 0.5, 0.75, 1.0, and 1.25 g of ginger powder, cardamom powder, and black pepper powder. Brahmi tisane without any spices was considered as control. Tisane was prepared by dipping in hot water for 3 min and sensory evaluation was done. Brahmi with 1 g ginger was having the highest sensory scores of 7.29 ± 0.18 (colour), 8.00 ± 0.31 (aroma), 7.86 ± 0.34 (taste), 7.71 ± 0.29 (after taste), and 7.71 ± 0.12 (overall acceptability). Tisane with 1 g ginger was significantly different from other combinations in case of taste, after taste, and overall acceptability (Table 3). Ginger- Brahmi tisane was evaluated and sensory scores revealed that it was acceptable at all the levels of ginger addition. The addition of ginger caused colour change in the product. As the weight of ginger increased, the whiteness in the product increased (Fig. 1A). Brahmi with 1 g ginger was showing the highest acceptability, while Brahmi with 1.25 g ginger showed a good score in the case of aroma, however it was minimal in colour and after-taste scores.

The effect of the weight of cardamom on sensory attributes of Brahmi tisane was analyzed statistically (Table 3). Brahmi with 0.5 g cardamom was showed the highest acceptability with sensory scores of 7.14 ± 0.26 (colour), 7.57 ± 0.20 (aroma), 7.00 ± 0.31 (taste), 7.57 ± 0.20 (after taste) and 7.32 ± 0.19 (overall acceptability). Tisane with 0.5 g cardamom was significantly different from other combinations in terms of after taste and overall acceptability. Results showed that there was no significant effect of the weight of cardamom on colour of the tisane. Cardamom-Brahmi tisane was evaluated and sensory scores (Fig. 1B) revealed that prepared tisane was satisfactory at all the levels of cardamom. The addition of cardamom didn't cause a significant colour change, but it showed a significant effect on the aroma. As the weight of cardamom increased, its aroma increased. Brahmi with 0.5 g cardamom gained the highest scores for aroma, after taste, and overall acceptability. Further, the taste and colour of the other combinations were not largely altered.

Brahmi with 1.25 g pepper was exhibited the highest acceptability with sensory scores of 8.57 ± 0.20 (colour), 8.00 ± 0.22 (aroma), 8.14 ± 0.26 (taste), 8.43 ± 0.20 (after taste), and 8.29 ± 0.14(overall acceptability). Tisane with 1.25 g pepper was significantly different from other combinations in terms of aroma, taste, after taste, and overall acceptability (Table 3). The addition of pepper largely affects the colour of the tisane. Pepper improved the colour of the Brahmi tisane. It also showed the effect on aroma, taste, and after taste of the tisane meaningfully up to 1.25 g addition. Brahmi with 1.25 g pepper

Table 3 Effect of amount of ginger, cardamom and black pepper on sensory attributes of Brahmi tisane

Amount of Ginger	Colour		Aroma		Taste		After taste		Overall acceptability	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Control	6.14[b]	0.26	5.57[c]	0.20	5.86[c]	0.26	6.43[b]	0.20	6.00[b]	0.14
0.5 g	7.14[a]	0.26	6.14[c]	0.26	6.00[b]	0.31	6.43[b]	0.20	6.43[b]	0.15
0.75 g	7.14[a]	0.26	6.57[c]	0.20	6.71[b]	0.29	6.86[b]	0.26	6.82[b]	0.17
1 g	7.29[a]	0.18	8.00[a]	0.31	7.86[a]	0.34	7.71[a]	0.29	7.71[a]	0.12
1.25 g	6.14[b]	0.26	7.71[b]	0.29	6.86[b]	0.26	6.71[b]	0.29	6.86[b]	0.22
C.D	0.72		0.74		0.85		0.73		0.47	
SE(m)	0.25		0.26		0.29		0.25		0.16	
SE(d)	0.35		0.36		0.41		0.35		0.23	
C.V	9.67		9.94		11.64		9.69		6.38	
Weight of cardamom										
Control	7.29[a]	0.18	6.00[a]	0.38	6.00[a]	0.31	5.71[c]	0.29	6.25[c]	0.15
0.25 g	6.71[a]	0.18	5.29[a]	0.18	6.14[a]	0.26	6.43[b]	0.20	6.14[c]	0.13
0.5 g	7.14[a]	0.26	7.57[a]	0.20	7.00[a]	0.31	7.57[a]	0.20	7.32[a]	0.19
0.75 g	6.43[a]	0.20	7.00[a]	0.31	6.57[a]	0.20	6.71[b]	0.18	6.68[b]	0.16
1.0 g	7.43[a]	0.20	6.43[a]	0.20	7.00[a]	0.31	6.71[b]	0.29	6.89[b]	0.05
C.D	0.61		0.77		0.82		0.69		0.42	
SE(m)	0.21		0.27		0.28		0.24		0.15	
SE(d)	0.30		0.38		0.40		0.33		0.21	
C.V	7.89		10.90		11.36		9.43		5.75	
Weight of black pepper										
Control	6.57[c]	0.20	6.14[b]	0.26	6.43[b]	0.20	6.43[b]	0.20	6.39[c]	0.12
0.75 g	7.57[b]	0.20	6.43[b]	0.20	6.43[b]	0.20	6.43[b]	0.20	6.71[c]	0.10
1 g	7.71[b]	0.18	6.57[b]	0.20	7.43[b]	0.20	6.14[b]	0.26	6.96[c]	0.13
1.25 g	8.57[a]	0.20	8.00[a]	0.22	8.14[a]	0.26	8.43[a]	0.20	8.29[a]	0.15
1.5 g	8.71[a]	0.18	7.57[a]	0.20	7.00[b]	0.22	6.43[b]	0.20	7.43[b]	0.14
C.D	0.57		0.63		0.63		0.62		0.37	
SE(m)	0.20		0.29		0.29		0.22		0.13	
SE(d)	0.28		0.31		0.31		0.30		0.18	
C.V	6.60		8.32		8.15		8.40		4.76	

Values in columns with different superscripts with small letters differ significantly ($p < 0.05$)

acquired the highest scores during sensory evaluation in terms of aroma, taste, after taste, and overall acceptability (Fig. 1C). In the case of colour, it was statistically similar to the sample with 1.5 g which got the highest score in colour. In herbal tea developed using *Zingiber officinale*, Rose (ginger) and *Pavetta crassipes* K SCHUM blends, total phenols, alkaloid content, and magnesium content was increased as the weight of ginger increased (Alakali et al. 2016). It also gave the 60/40 ratio of the product blend of Ginger/pavetta having the highest score for its overall acceptability (Alakali et al. 2016). Herbal tea preparation from mulberry leaves with three different combinations of tulsa (4:1), ashwagandha (3:2), and only mulberry leaves as a control. However, all three combinations were having bitter odour and taste (Killedar and Pawar 2017). Optimized powder of 3 g was filled in a dip bag and herbal tea was prepared by infusing in 150 ml of boiling water for 3 min for guava leaf herbal tea preparation. Herbal tea combination of 75% guava leaves, 15% coriander leaves, ginger, and 10% lemongrass was accepted with the best sensory score (Akila et al. 2018). Polyherbal tea from antioxidant-rich medicinal herbs formulation containing 15% *M. pumilum*, 19% *M. citrifolia*, 10% *C. sinensis,* 10% *H. sabdariffa*, 45% *C. asiatica*, and 1% *S. rebaudiana* exhibited the highest mark for overall acceptability (Nasir et al. 2019).

Evaluation of developed tisane combinations

Optimized combinations of ginger-Brahmi tisane, cardamom-Brahmi tisane, and pepper-Brahmi tisane were compared (Fig. 2). Without any spices, sole Brahmi was considered as control. Ginger-Brahmi (1:1.5), cardamom-Brahmi (0.50:1.5), pepper-Brahmi (1.25:1.5), and Brahmi (1.5)

Table 4 Effect of different spices on sensory attributes of Brahmi tisane

Tisane	Colour		Aroma		Taste		After taste		Overall acceptability	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Control	5.71^{d}	0.29	5.29^{c}	0.29	5.57^{c}	0.37	5.86^{d}	0.26	5.61^{c}	0.26
Ginger	7.57^{b}	0.20	7.71^{a}	0.29	6.71^{b}	0.29	7.29^{c}	0.18	7.32^{b}	0.13
Cardamom	6.86^{c}	0.26	6.86^{c}	0.26	7.43^{b}	0.30	$8.00^{\mathbf{b}}$	0.29	7.29^{b}	0.15
Pepper	8.71^{a}	0.18	8.46^{a}	0.20	8.43^{a}	0.20	8.29^{a}	0.18	8.46^{a}	0.10
C.D	0.70		0.77		0.87		0.63		0.50	
SE(m)	0.24		0.26		0.30		0.21		0.17	
SE(d)	0.34		0.37		0.42		0.30		0.24	
C.V	8.69		9.76		11.08		7.71		6.24	

Values in columns with different superscripts with small letters differ significantly ($p < 0.05$)

Table 5 Phytochemical composition of Brahmi tisane

Parameter	Amount (per 100 ml)
Bacoside A (mg)	0.036 ± 0.0004
TAC (mg TE)	87.72 ± 1.38
TPC (mg GAE)	106.02 ± 1.03
Calcium (µg)	21,100 ± 3.08
Iron (µg)	87 ± 0.9

Values are Mean ± SD of 3 replications

were filled in dip bags, and tisane was prepared by dipping in hot water for 3 min. Prepared tisane was evaluated and compared among them. Among three combinations, pepper obtained the highest acceptability score with 8.71 ± 0.18 (colour), 8.43 ± 0.20 (aroma), 8.43 ± 0.20 (taste), 8.29 ± 0.18 (after taste), and 8.46 ± 0.10 (overall acceptability). All the combinations including control were significantly different in every attribute except after taste (Table 4). This may be because of the taste of Brahmi which is the main reason for after taste attribute. Pepper- Brahmi tisane was having good colour compared to other combinations. In terms of taste, pepper and ginger tisane showed higher acceptability as compared to cardamom and control. Ginger tisane also gained next to higher scores in the after-taste attribute. In case of aroma, cardamom got a higher score along with pepper. Herbal tea composed of 50% Moringa, 30% roselle, and 20% lemongrass, was the most preferred in colour, flavour, astringency, and overall sensory properties while the control made up of 100% Moringa brewed the least preferred herbal tea in most of the sensory attributes (De-heer et al. 2013).

Phytochemical estimation of brahmi tisane

Optimized and overall accepted Brahmi tisane was subjected to its phytochemical analysis (Table 5). Bacoside A was found around 0.036 mg/100 ml from an optimized amount of 1.5 g dip bag, which is very less compared to methanolic extracts of dried Brahmi (~ 28 mg/g). Maximum bacoside-A content in Jal brahmi (DBM-4) was observed a 3.65% W/W on a dry weight basis (Gubbannavar et al. 2013; Saesong et al. 2019; Saran et al. 2022). This may be due to the poor solubility of Bacoside A in water. The total Antioxidant content (TAC) of overall acceptable tisane was 87.72 mg TE/100 ml. This indicated that Brahmi tisane was also having a good amount of antioxidant capacity like green tea. Therefore, Brahmi tisane can be suggested as a replacement for green tea. Analysis showed that Brahmi tisane contains 106.02 mg GAE/ 100 ml of TPC (Table). The Total Phenolic content of green tea infusion is 116 mg GAE/ml (Yang and Liu 2013) From the formulation of polyherbal tea from antioxidant-rich medicinal herbs such as *Camellia sinensis, Hibiscus sabdariffa, Marantodes pumilum, Morinda citrifolia, Centella asiatica* and *Stevia rebaudiana, a* single component of *S. rebaudiana* showed the highest total phenolic (TPC) and total flavonoid content (TFC). The mixture of the above six selected herbs consisted significant weight of TPC (198.18 ± 0.02 mg GAE/L) and TFC (192.07 ± 0.03 mg RE/L infusion (Nasir et al. 2019). In the present study, it was found that tisane contains 21,100 µg/100 ml of calcium and 87 µg/ 100 ml of iron which are essential minerals in bone development, healthy muscles, and the circulatory system. An herbal tea preparation from *Zingiber officinale*, represented that the total phenols, alkaloid content, and magnesium content were increased as the weight of ginger increased (Alakali et al. 2016). Herbal tea was prepared with tulsa (4:1), ashwagandha (3:2), and only mulberry leaves showed the presence of alkaloids, glycosides, flavonoids, and tannins in the tea (Killedar and Pawar 2017). An herbal tea containing *M. oleifera*, *Z. officinale,* and *C. limon* was successfully formulated with good physicochemical properties and antioxidant activity (Builders et al. 2020). Therefore, Jal brahmi tisane can be chosen for health benefits as compared to green tea.

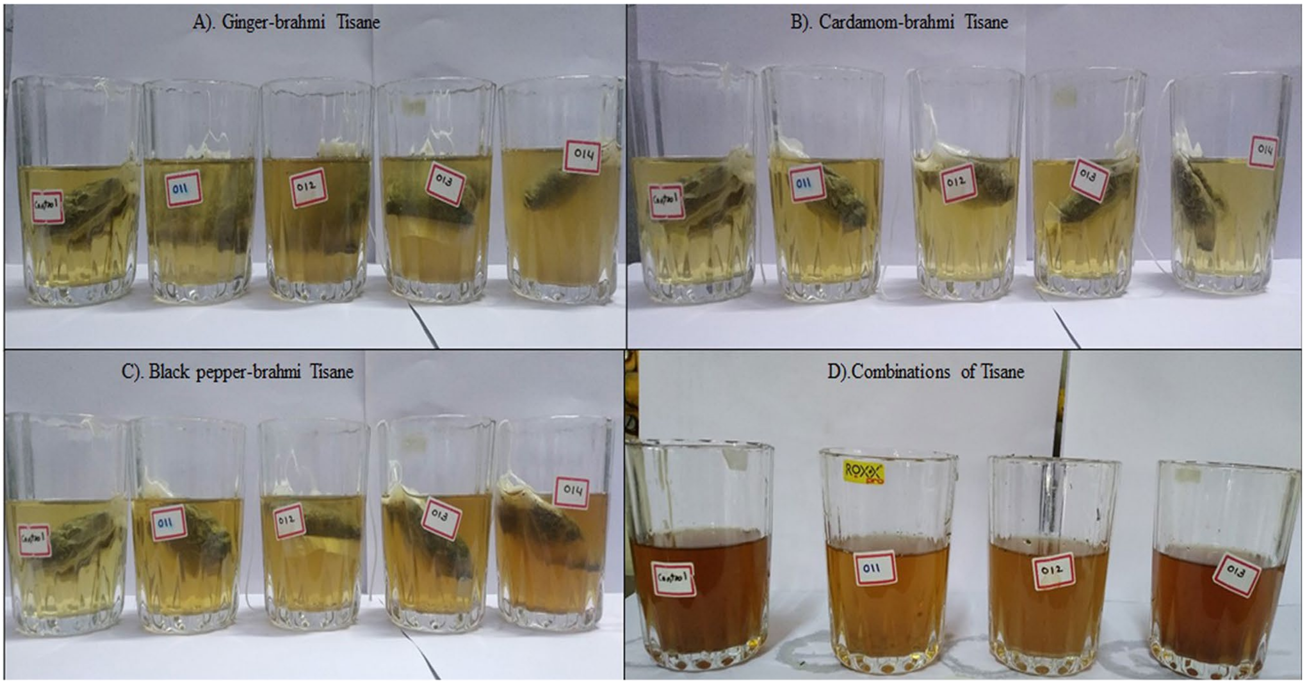

Fig. 2 Brahmi tisane prepared with different spice combinations

Conclusion

Indigenous herbs like Jal brahmi are important for human health due to their huge dietary potential. In the composition of Jal brahmi tisane, a weight of 1.5 g dried Brahmi per dip bag with a dipping time of 3 min is recommended with the highest sensory scores for best tisane formulation. Additionally, pepper-Brahmi tisane was selected as it scored higher for all the sensory attributes as compared to cardamom, ginger, and control (without spices). Jal brahmi is a good source of antioxidants, phenolic compounds, and saponins especially Bacosides which are responsible for the brain tonic effect. Therefore, it can be considered one of the best options to replace green tea as it is also free from caffeine. Further, there is a need to explore the potential of indigenous herbs for the development of novel herb tea.

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Authors' contribution PGH conceived, performed, interpreted the experiments data recording, tables and figure's preparation. GRJ performed chemical analysis and product development. KBK designed and analyse the experiment and editing work. HID and PLS were performed collection, multiplication, conservation of germplasm. They also write and processed the manuscript.

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Code availability No code availability.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the manuscript.

Ethical approval Manuscript does not involve any biological studies of any animal or human data "Not applicable".

Consent to participate All authors have read and agreed for the publication of the MS.

Consent for publication The manuscript does contain any individual person's data in any form (including any individual details, images or videos).

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