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Development, Analysis and Evaluation of Herbal Tea from *Neolamarckia Cadamba* Leaves Infused with Tulsi Leaves

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ABSTRACT

This research paper explores the development of a unique herbal tea blend using *Neolamarckia cadamba* (NC) leaves and Tulsi leaves with cardamom flavor. Herbal teas have gained popularity due to their potential health benefits and unique flavor profiles. Herbal tea powder variants derived from carefully selected ingredients renowned for their medicinal properties and phytochemical profiles. The process involved drying leaves for pre-determined duration, followed by fine crushing and the addition of cardamom for flavor enhancement. Three variants were developed by altering the proportions of raw materials and subsequently subjected to sensory evaluation using a 9-point hedonic scale, assessing attributes such as colour, appearance, aroma, taste, and overall acceptability. Results indicated that the variant with equal proportions of raw materials garnered the highest sensory scores. Proximate, mineral, and phytochemical analyses were conducted, revealing moisture content, crude fiber content, and total ash content at 1.23%, 8.25%, and 9.2%, respectively. Calcium (Ca) and Iron (Fe) were present at 16.025 mg/2g sample and 32.535 mg/2g sample, respectively. Phytochemical analysis demonstrated ascorbic acid and total antioxidant activity at 22.58 mg/100g and 513.11 mg AEAC/100g dry sample, respectively. The herbal tea powder exhibited high flavonoid and total phenol content ranging from 5484.76 to 5621.81 mg Quercetin/100g and 924.67 to 928.27 mg GAE/100g, respectively. Consequently, it is recommended as a beverage offering significant health benefits.

Keywords : Phytochemicals, Antioxidant properties, Health benefits, Mineral analysis, Sensory evaluation

INTRODUCTION

Herbal tea is a mixture of leaves, seeds, and/or roots from different plants. Many types of herbal teas have been utilized for their therapeutic qualities. A portion of them is consumed for their stimulating qualities, which aid in relaxation, relieve gastrointestinal distress, and boost the immune system. Herbal teas such as black, green, chamomile, ginger, ginseng, peppermint, cinnamon, and so on are quite popular. Several of these herbal drinks have incredibly potent therapeutic effects (Ravikumar, 2014).

Herbal teas are free of caffeine, in contrast to most other types of tea. They're also quite tasty and convenient to drink. The majority of herbal teas are made with one primary herbal component or a combination of herbs, each of which is meant to achieve a particular goal. Among them, relaxation, rejuvenation, or alleviation from a certain ailment (Aoshima *et al.*, 2007).

Over 50,000 medicinal plants exist that are the richest source of bioactive components and have substantial nutritional value, yet they are currently underutilized in the food industry. *Neolamarckia cadamba* is one of those medicinal plants, and it has several health benefits, such as an antidiabetic, anti-inflammatory, antioxidant, analgesic, etc. Tulsi is also used for its medicinal values and to increase the health benefits of herbal tea. This study highlights all the essential nutrients present in the developed herbal tea powder, making it useful in food preparation.

Neolamarckia cadamba, also known as Kadam, is a tropical tree native to Southeast Asia with antioxidant

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and anti-inflammatory properties. *Neolamarckia cadamba* (family: Rubiaceae) is a tree that is traditionally used for the treatment of various illnesses. Numerous pharmacological qualities of this plant's leave have been demonstrated, including antioxidant (Chandel *et al*., 2011), antidiabetic (Ahmed *et al.*, 2011), antitumor (Dolai *et al.*, 2012), anti-inflammatory, antipyretic, analgesic (Mondal *et al.*, 2009), antimicrobial (Rafshanjani *et al.*, 2014), and anticancer effects (Singh *et al.*, 2013).

Tulsi, a revered herb in Ayurveda, is known for its adaptogenic and immune-boosting properties. Tulsi has been recognized as a medicinal herb for curing various diseases such as diarrhoea, dysentery, skin irritation and bronchitis (Pattanayak *et al.,* 2010). The leave extract of this plant has been reported to possess antidiabetic, anticancer, antimicrobial, and cardioprotective activities, and as such, is of used as traditional medicine (Barah and Biswas, 2018). Regular use of Tulsi-leaf extract has been shown to prevent illnesses and improve overall health and well-being.

Cardamom, a spice with aromatic and digestive benefits, is often combined with these leaves to create a unique herbal tea blend. Cardamom contains volatile oils, flavonoids, and phenolic compounds, which are anti-inflammatory and antioxidants. Research Afrina *et al.* (2016) showed that cardamom extract contains alkaloids, saponins, tannins, polyphenols, flavonoids, quinones, steroids, and triterpenoids. Fachriyah and Sumardi (2007) stated that the components of cardamom seed essential oils are α-pinene, β-pinena, p-simene, 1.8 sineol, and α-terpineol. Flavonoid and phenolic compounds found in cardamom are also potential as antioxidants and antidiabetic.

Based on these above scientific findings the present course of study aims at development of herbal infusions of *Neolamarckia cadamba* and Tulsi leaves by blending with the cardamom and subsequently phytochemical analysis, and deciphering their antioxidant activity was done.

The combination of these leaves with cardamom, a spice with aromatic and medicinal qualities, will result in a synergistic herbal tea experience.

Objectives:

1. To formulate an herbal tea blend using *Neolamarckia cadamba* leaves, Tulsi leaves, and cardamom flavor.
2. To analyze the sensory attributes of the herbal tea.
3. To conduct proximate analysis, mineral analysis and phytochemical composition of the developed product.

METHODOLOGY

Collection of Ingredients:

Neolamarckia cadamba leaves and Tulsi leaves were sourced from the Medicinal Botanical Garden of the Ayurveda Department of Parul University, Vadodara, Gujarat, India, and cardamom was obtained from a local market in Vadodara.

Drying of leaves:

The collected leaves were sorted and washed with clean water. Prior to drying in the tray dryer, the leaves were air dried. Kadam leaves were dried at 60°C for 5 hours, while Tulsi leaves were dried at 50°C for 3 hours in a tray dryer.

Preparation of herbal tea:

Dried leaves were manually crushed separately, and cardamom seeds were removed from pods and crushed to extract flavor. Crushed materials are blended in three different quantities to determine the sensory properties of each variation. The first formulation combines dried *Neolamarckia cadamba* leaves in a higher ratio than Tulsi leaves and cardamom. The second formulation has the Tulsi leaves in a higher ratio, while another formulation contains leaves and cardamom in equal proportions. The mixture is placed into a tea bag. Each tea bag contained 2 grams of herbal tea powder blend. The mixture was soaked in boiling water between. 80-90^{0}C for 3-4 minutes (Table 1).

Table 1 : Formulation of Herbal tea powder

Ingredients	S_1	S_2	S_3
Neolamarckia cadamba leaves (g)	1 gram	0.5 gram	0.7 gram
Tulsi leaves (g)	0.5 gram	1 gram	0.7 gram
Cardamom (g)	0.5 gram	0.5 gram	0.7 gram

Sensory Evaluation:

Faculty lecturers from Parul University's Food Technology Department in Vadodara, Gujarat, India, conducted a sensory evaluation of the herbal tea blend to determine its flavor, aroma, appearance, colour, and acceptance. The sensory properties were evaluated using

a 9-point hedonic scale scoringsystem. The herbal tea with an equal proportion of *Neolamarckia cadamba* leaves, Tulsi leaves, and cardamom had the highest sensory score.

Comprehensive proximate, mineral and phytochemical analysis:

The formulation of herbal tea powder which has highest sensory score herbal tea powder underwent analysis to determine its proximate composition, mineral content, and phytochemical constituents, aiming to quantify both its nutritional content and the levels of bioactive compounds present.

Proximate analysis:

The Association of Official Analytical Chemists (AOAC) method was used to conduct proximate analysis of the herbal tea blend. Herbal tea powder was weighed in a petri plate and dried in an oven at 105^0C to determine moisture content. The percentage moisture was estimated based on weight loss. Ash content was assessed using a muffle furnace at 550^0C for 5 hours. The crude fibre content was assessed using the AOAC 978.10 technique.

Mineral analysis:

5ml of ash solution was diluted with water and HCl in a 1:1 ratio. The beaker is then placed in a water bath (with a lid) for 30 minutes. After that, the lid is rinsed with water and HCl in a 1:1 ratio and returned to the water bath (uncovered) for another 30 minutes. The solution is filtered after adding 10 ml of water and HCl at a 1:1 ratio, and the volume has been increased to 100 ml with the same HCl and water ratio. This ash solution was used to estimate calcium and iron. Calcium was determined using the titrimetric method, while iron was determined using U.V. spectrophotometer.

Phytochemical analysis:

Determination of Ascorbic acid:

In this experimental procedure, an indophenol solution is standardized using a standard ascorbic acid solution. The standardized solution is then used to estimate the ascorbic acid content in an unknown solution. First, a known quantity of standard ascorbic acid solution is titrated against the indophenol solution until a pink colour persists for 5 seconds, indicating the endpoint of the titration. The volume of indophenol solution used in the titration corresponds to the amount of ascorbic acid present in the standard solution. To estimate the ascorbic acid content in the unknown solution, the sample is homogenized with a 4% oxalic acid solution, filtered, and diluted. The diluted extract is then titrated against DCPIP (2,6-dichlorophenol indophenol), and the vitamin C content is calculated as milligrams of ascorbic acid equivalents per 100 grams of fresh weight using a standard curve of L-ascorbic acid. This method allows for the quantitative determination of vitamin C in the sample.

Formula for calculating Vitamin C:

Vitamin C (mg/100g) = Titre value × Standard value (µg) × Total Vol of extract / Assay volume × Weight of the sample (g) × 1000

Antioxidant activities of herbal tea powder as determined by DPPH radical scavenging method:

In this experimental procedure, a sample is weighed and incubated in methanol (80%) for 72 hours to extract its antioxidant compounds. The extract is then homogenized and mixed with acetate buffer and DPPH solution. After incubation, the absorbance of the solution is measured spectrophotometrically at 517 nm (A1). The absorbance of DPPH solution without the sample is also measured (A2). The difference in absorbance between the sample and the blank (A2 - A1) is calculated to determine the decrease in absorbance due to the antioxidant activity of the sample. A standard curve using different concentrations of ascorbic acid is developed to quantify the antioxidant capacity of the sample, expressed as ascorbic acid equivalent antioxidant capacity. The concentration of ascorbic acid per unit absorbance (µg/OD) is determined by dividing the concentration of ascorbic acid by the difference in absorbance. Finally, the antioxidant concentration in the sample extract (µg/ml) is calculated by multiplying the absorbance of the sample by the difference in absorbance per unit absorbance. This method provides a quantitative assessment of the antioxidant activity of the sample extract.

Formula for calculating Radical scavenging activity (RSA):

RSA = Absorbance of control – Absorbance of sample / Absorbance of control × 100

Determination of Total phenols:

In this experimental procedure, the total phenolic content is estimated using the Folin-Ciocalteu (FC) method. Firstly, a standard calibration curve is prepared

by pipetting out standard Gallic acid solutions into labelled test tubes, ranging from 20 to 100 µg. FC reagent and distilled water are added to each test tube, followed by the addition of Na_2CO_3 solution after 10 minutes. After allowing the solution to stand for 60 minutes at room temperature, the absorbance of the blue colour formed is measured at 750 nm using a spectrophotometer, and a standard calibration curve is plotted by correlating absorbance with the concentration of Gallic acid.

For the extraction and estimation of total phenols from the sample, 5g of the sample is weighed and incubated in methanol (80%) for 72 hours, followed by homogenization and pooling of the extracts. A portion of the extract is then mixed with FC reagent, distilled water, and Na_2CO_3 solution in test tubes. After 10 minutes of incubation, the absorbance of the blue colour formed is measured at 750 nm using a spectrophotometer. The absorbance obtained is then compared with the standard calibration curve to determine the total phenolic content in the sample.

Formula for calculating Total phenol:

Total phenol content (mg gallic acid equivalents/100g) = OD700nm × Std. value (µg/OD) × Total Vol. of extract × 100 / Assay volume × Wt. of sample (g) × 1000

Determination of Total flavonoids:

The colorimetric technique with aluminium chloride was used to assess the total flavonoid content. At an alkaline pH, flavonoids react with $AlCl_3$ and $NaNO_2$ to produce a brick red colour. At 510 nm, the complex's absorbance is measured. The basic idea behind the AlCl3 colorimetric approach is that $AlCl_3$ combines with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols to generate acid stable complexes. Moreover, it combines with the orthodihydroxyl groups in the flavonoid A-or B-ring to generate acid-labile complexes.

This process involves conducting the complexation reaction in an alkaline media with $NaNO_2$. The nitration of any aromatic ring containing a catechol group with its third or fourth positions unsubstituted or not sterically hindered is the basis of the procedure. The combination generated a yellow solution upon the addition of Al (III), which promptly became red upon the addition of NaOH. The absorbance is measured at 510 nm.

Formula for calculating Total flavonoid content:

Total flavonoid content (mg Quercetin equivalents/100g) = Abs 510 nm × Std. value (µg/1 Abs) × Total Vol. of extract × 100 / Assay volume × Wt. of sample (g) × 1000

RESULTS AND DISCUSSION

Proximate analysis:

Proximate analysis of herbal tea powder depicts their nutritional value. Analysis comprises of determination of moisture (by oven drying method), crude fibre (by AOAC 978.10 method), and ash content (by ashing the sample in muffle furnace), of herbal tea powder is depicted in Table 2. Table 2, illustrates the result of proximate analysis of herbal tea powder prepared from *Neolamarckia cadamba* infused with Tulsi leaves. It can be stated that herbal tea powder is rich in crude fibre and ash content, 8.25%, and 9.2%, respectively. And it possesses a moisture content of 1.23%.

Table 2 : Results of Proximate analysis

Parameters	Quantity
Moisture (%)	1.23 %
Total ash (%)	9.2 %
Crude fiber (%)	8.25 %

Mineral analysis:

Calcium content in the herbal tea powder was determined via titrimetric method, where it was precipitated as calcium oxalate, filtered, washed, and dissolved in hot dilute H_2SO_4, followed by titration with standard potassium permanganate solution. The analysis indicated a calcium content of 16.025 mg/2g of sample. Iron content, determined using a UV spectrophotometer, was found to be high at 32.535 mg/2g of sample (Table 3).

Table 3 : Results of Mineral analysis

Parameters	Quantity
Calcium (mg/ 2g sample)	16.025 ± 1.13
Iron (mg/ 2g sample)	32.535 ± 4.37

Phytochemical Composition:

The herbal tea exhibited a rich phytochemical composition, with significant levels of total phenol, and flavonoids. Total phenol content was estimated by spectrophotometric method using Folin Ciocalteu reagent (FC). The colorimetric technique with aluminium chloride was used to assess the total flavonoid content. Antioxidant activities of herbal tea powder as determined by DPPH radical scavenging method (Table 4).

Table 1: Results of Phytochemical analysis

Parameters	Quantity
Ascorbic acid (mg/100g)	22.58 ± 1.29
Total Antioxidant activity (mg AEAC/ 100g)	513.11 ± 6.18
Total phenol (mg GAE/ 100g)	926.47 ± 2.54
Flavonoids (mg Quercetin/ 100g)	5553.29±96.90

Health benefits:

Literature review highlighted the potential anti-inflammatory, Antioxidant Properties, and immune-boosting effects associated with the consumption of *Neolamarckia cadamba*, Tulsi, and cardamom. The infusion of *Neolamarckia cadamba* and Tulsi leaves enhances the medicinal value of the herbal tea, while cardamom adds a distinctive flavor profile. Consumption of *Neolamarckia cadamba* and Tulsi leaves may help boost immunity, reduce inflammation, and alleviate stress. Cardamom is known for its digestive benefits and may aid in gastrointestinal health. The antioxidant properties of the herbal tea blend contribute to overall health and well-being.

Conclusion:

An herbal tea blend combining *Neolamarckia cadamba* leaves, Tulsi leaves, and cardamom flavor has been developed, offering a natural and healthy beverage option. The blend combines the medicinal properties of Neolamarckia cadamba and Tulsi with the aromatic richness of cardamom. The potential health benefits of this herbal tea are being explored, and further research is needed to refine the formulation and conduct clinical studies to validate the health claims associated with the tea. This herbal tea blend is a valuable addition to the growing market of functional beverages.

Future directions:

Further research is needed to understand the long-term health benefits of regular consumption of the herbal tea, as well as to determine optimal brewing conditions and formulation variations. Future directions include examining the tea blend's shelf life, conducting clinical trials to validate its health benefits, and exploring ingredient proportions and infusion methods to optimize flavor and health benefits.

Abbreviations:

NC- *Neolamarckia cadamba*

mg- Milligram

AEAC- Ascorbic acid Equivalent Antioxidant Content

GAE- Gallic Acid Equivalent

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