Journal of Plant Resources (2022) **20**(1), 139-149
https://doi.org/10.3126/bdpr.v20i01.56603

Formulation of Herbal Tea from Nepalese Medicinal Plants: Phenolic Assay, Proximate Composition and In-vivo Toxicity Profiling of Medicinal Plants with Nutritive Benefits

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Abstract

Herbal tea, also known as tisane, is a beverage made from the infusion or decoction of plant material in hot water. True tea comes from the *Camellia sinensis* plant, while tisane (herbal tea) comes from a water-based infusion of herbs, spices, flowers, leaves etc. This study aimed to formulate and determine the nutraceutical value (proximate analysis), phytochemical value (total phenolic content) and in-vivo toxicity of the different medicinal plants used to prepare three different types of herbal tea formulations. Natural Product Research Laboratory (NPRL)-1 [*Asparagus officinalis* L., *Phyllanthus emblica* L., *Mentha piperita* L., *Elettaria cardamomum* (L.) Maton and *Camellia sinensis* (L.) Kuntze], NPRL-2 [*Ocimum tenuiflorum* L., *Bergenia ciliate* (Haw.) Sternb., *Elettaria cardamom* (L.) maton and *Camellia sinensis* (L.) Kuntze] and NPRL-3 [*Salvia rosmarinus* Spenn., *Cymbopogon citratus* (DC.) Stapf, *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. *Elettaria cardamomum* (L.) Maton and *Withania somnifera* (L.) Dunal] herbal tea formulations were prepared from these selected medicinal plants. They were respectively tested for their properties. All the plants included were highly nutritional and none were found toxic. The results suggested that herbal tea made up of these potent plants' parts can be a good choice for health-promoting benefits. These formulations could further be studied for their other beneficial activities.

Keywords: Acute oral toxicity, *Asparagus officinalis*, Phytochemical screening, Proximate analysis

Introduction

Since the dawn of humankind, various plants and plant-derived products have been used as medicine. According to the Biodiversity Profile Project (1995), Nepal ranks ninth among Asian countries in floral diversity, with approximately 9,000 flowering plants. Catalogs of Nepal have recorded 1,792 (Rokaya et al., 2010) to 2,331 (Baral & Kurmi, 2006) useful medicinal and aromatic plants, reporting their significance in mitigating human suffering due to their prolonged use in daily lives as home remedies and traditional therapies (Kunwar et al., 2013).

Herbal tea, prepared by infusing herbs in hot water, offers numerous health benefits. Bioactive compounds and phytochemicals in tea have therapeutic effects, particularly in preventing metabolic diseases like diabetes and obesity. Polyphenol in herbal tea consumption, with antioxidant, anti-inflammatory, and antimutagenic properties, alleviates numerous chronic disease (Arts & Hollman, 2005). Additionally, herbal tea is rich source of vital macronutrients which paly indispensable roles in physiological and metabolic process crucial for optimal health. Medicinal plants have gained the sufficient attention in recent year for their potential use in pharmaceutical, nutritional supplements and other health promoting products due to their presumed safety, nutritional value and therapeutic effects (Alali et al., 2021).

Asparagus officinalis (Kurilo) confers significant health benefits during pregnancy, including enhancing milk production and improving its quality, alleviating indigestion acidity disorders, and bolstering immunity in both mother and baby (Kumar et al., 2014). Multitude of research have revealed that asparagus shows galactagogue

and mammogenic function by increasing blood prolactin and cellular division in the mammary gland increasing lactation (Aryal et al., 2017; Birla et al., 2022; Dahiya et al., 2022; Tanwar et al., 2008). Its root powder has traditionally been used and distributed to lactating mothers in various parts of Nepal (Liu et al., 2015). A study conducted by Nepal Health Research Council (NHRC) in 2017 to assess the effectiveness of the *Asparagus* powder by distribution program on breastfeeding promotion revealed the Asparagus powder improved the health of women, enhanced breast milk production, reduces back pain and improves the child health (Aryal et al., 2017).

Similarly, *Phyllanthus emblica*, *Mentha piperita* and *Elettaria cardamomum* are noted to boost the efficacy and flavor of the tea. Amala has been extensively studied in vivo and shown to possess hypoglycemic, anti-inflammatory, anti-hyperlipidemic and antioxidant properties (Kapoor et al., 2020). *Bergenia ciliata* has been used in folk medicine to treat diabetes mellitus symptoms. In rats treated with streptozotocin (STZ), extracts of the root and leaves of *Bergenia ciliata* were found to have hypoglycemic action (Islam et al., 2002). It is further reported to possess antioxidant, anti-inflammatory, antitussive, anti-ulcer and anti-neoplastic properties with antifungal, antiviral, antiplasmodial and antibacterial properties (Ahmad et al., 2018; Koul et al., 2020).

Ocimum tenuiflorum leaf extract also significantly lowers blood glucose levels in glucose-induced hyperglycemic and STZ-induced diabetic rats (Chattopadhyay, 1993). Moreover, the chemical constituents in *Ocimum tenuiflorum*, such as oleanolic acid, eugenol, linalool, rosmarinic acid and β caryophyllene contribute to the diuretic and stimulant property (Panchal & Parvez, 2019). Rosemary and its compounds have also been studied for a wide range of medicinal properties (Andrade et al., 2018; Rahbardar & Hosseinzadeh, 2020). It has been reported that rosemary improves memory, reduce anxiety and depression and improve sleep quality (Nematolahi et al., 2018).

Similarly, frequently used in aromatic therapy, lemongrass is believed to have some perceived anxiolytic effects (Goes et al., 2015). It is statistically proven that inhaling lemongrass essential oil may improve cognitive function and mood in healthy women while not affecting physiological status (Sriraksa et al., 2018). Likewise, Ashwagandha's root extract is proven to improve sleep quality and help with insomnia management (Langade et al., 2021).

The current study attempted to use the above-mentioned plants with high medicinal value in Herbal tea formulation after analyzing theirs in-vivo toxicity, phytochemical assessment and nutraceutical value.

Materials and Methods

Plant collection and extraction techniques

The medicinal plants selected for the preparation of herbal formulation is of Nepali origin and documented for medicinal values (Table 1). The plants were collected from local vendors keeping in mind that the ordered plants are of high quality and collected on time.

Aqueous maceration was used to extract the crude extract of each constituent of herbal tea for phytochemical analysis, test for acute oral toxicity and quantification of phenolic and flavonoid content. The ground powder was used for the proximate analysis.

Three different formulations were prepared based on their nutritional value: NPRL-1, NPRL-2 and NPRL-3.

NPRL-1

The first formulation (NPRL-1) included *Asparagus officinalis* L. (Kurilo), *Phyllanthus emblica* L. (Amala), *Mentha piperita* L. (Mentha), *Elettaria cardamomum* (L.) Maton (Elaichi) and *Camellia sinensis* (L.) Kuntze (Tea).

NPRL-2

The second formulation (NPRL-2) was aimed to provide a refreshing sensation to our body, especially while feeling stressed. The plants used for this formulation were *Salvia rosmarinus* Spenn. (Rosemary), *Cymbopogon citratus* (DC.) Stapf (Lemon Grass), *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. (Khayar Bark), *Elettaria cardamomum* (L.) Maton (Elaichi) and *Withania somnifera* (L.) Dunal (Ashwagandha).

NPRL-3

The third formulation (NPRL-3) was formulated with *Ocimum tenuiflorum* L. (Tulsi) and *Bergenia ciliata* (Haw) Sternb. (Pakhanbed) as the main ingredients. Meanwhile, tea leaves (*Camellia sinensis*) and Elaichi (*Elettaria cardamomum*) were added for flavour and colour. NPRL-3 was formulated keeping the benefits of these medicinal plants in regard, especially to work as a potential anti-diabetic source.

Preparation of herbal tea

The preparation of herbal tea starts with separating the healthy parts of the sample collected. The sorted materials were manually cleaned and cut into small pieces. The sample was dried at 35-40°C. After achieving optimum dryness, it was sieved for size uniformity. The specified constituents were thoroughly mixed and packed in an airtight polybag pouch.

Proximate analysis of herbal tea samples

Proximate analysis was performed on the herbal tea samples to determine the moisture content, fat, crude protein, fiber, total ash and total carbohydrate using AOAC, 18th edition official method (Horwitz & Latimer, 2005). Energy value (Kcal/100g) was calculated based on their crude protein, fat, and carbohydrate content using the formula described by Crisan and Sands (1978).

Phytochemical screening

The presence of alkaloids, glycosides, flavonoids, tannins, phenols, saponins, carbohydrates and steroids were identified for qualitative screening of phytochemicals of aqueous extracts of each constituent of herbal tea formulation using the standards method (Evans, 2009).

Determination of total phenolic content

The total phenolic content of constituents of herbal tea was determined by using the Folin-Ciocalteu method taking Gallic acid as standard for the calibration curve as described by Singleton and Rossi (1965) with a little modification.

Standard Gallic acid was prepared by dissolving 0.500 grams of dry gallic in 10 ml ethanol and diluting it to 100 ml using distilled water. To prepare the calibration curve, various concentrations of the solution were prepared. From each calibration solution, 2 µL gallic acid was pipetted in a triplicate manner and mixed with 158 µL distilled water and

Table 1: List of medicinal plants used for formulation

Types of Tea	Common Name	Scientific Name	Family	Part Used
NPRL-1	Kurilo	*Asparagus officinalis* L.	Asparagaceae	Root
	Amala	*Phyllanthus emblica* L.	Phyllanthaceae	Fruit
	Mentha	*Mentha piperita* L.	Lamiaceae	Leaves
	Elaichi	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Fruit
	Tea leaf	*Camelia sinensis* (L.) Kuntze	Theaceae	Leaves
NPRL-2	Rosemary	*Salvia rosmarinus* Spenn.	Lamiaceae	Leaves
	Lemon Grass	*Cymbopogon citratus* (DC.) Stapf,	Poaceae	Leaves
	Khayar Bark	*Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	Bark
	Ashwagandha	*Withania somnifera* (L.) Dunal	Solanaceae	Root
	Elaichi	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Fruit
NPRL-3	Tulsi	*Ocimum tenuiflorum* L.	Lamiaceae	Leaves
	Pakhanbed	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Rhizomes
	Tea Leaf	*Camelia sinensis* (L.) Kuntze	Theaceae	Leaves
	Elaichi	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Fruit

10 µL Folin–Ciocalteu reagent (FCR) (10%) in a 96-well plate. The mixture was then left for 8 minutes in an atomized shaking mode of EPOCH 96 well plate reader. Then, the initial reading was taken at 765 nm. 30 µL Na_2CO_3 was added to each well containing the previous solution and was incubated for 30 minutes at 40°C, and the final reading was taken. All the experiments were carried out in triplicate and the average absorption value obtained at different concentrations of gallic acid was used to plot the standard curve (Figure 1).

5000 ppm concentration of extracts was prepared. The procedure as described for standard Gallic acid was followed and absorbance for a specified concentration of the extract was determined. The sample was loaded in triplicate manners for experiments.

The total phenolic content was determined using the formula below:

Gallic Acid Equivalent (GAE) =

$$\frac{\text{Absorbance at 765 nm} + \text{C(from calibration curve)}}{\text{Slope of calibration curve (m)}}$$

Total Phenolic Content (mg GAE/g) =

$$\frac{\text{mg GAE}}{\text{extract concentration (g)}}$$

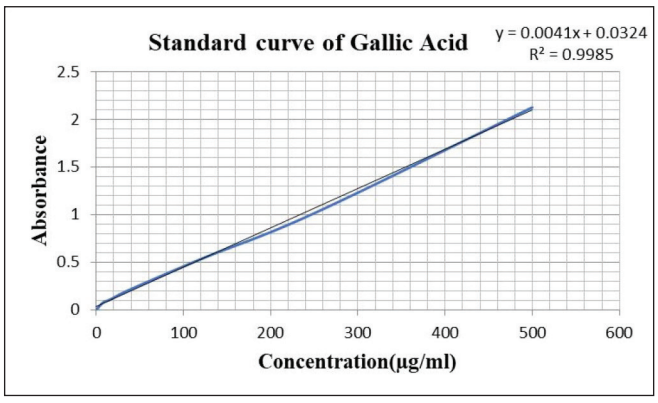

Figure 1: Standard calibration curve of Gallic acid

Acute oral toxicity

Acute oral toxicity is based on the principle that the toxicity of a chemical or plant extracts may be assessed with a small number of animals and adequate information on the acute toxicity of the test material can be obtained to facilitate classification. Acute oral toxicity of the extracts was performed according to the OECD guideline 423 for testing of chemicals acute oral toxicity-acute toxic class method (Organisation for Economic Co-operation and Development [OECD], 2002). For the test, nulliparous and non-pregnant Swiss albino mice of age between 8 and 12 weeks old were used, which were kept in the animal house of Natural Product Research Laboratory, Thapathali, Kathmandu. The mice were selected randomly, labeled to ensure individual identification, and kept in their cages for at least one day before dosing to allow for acclimation to laboratory settings. Before dosage, animals have fasted for 24 hrs.

The extracts were weighed at a dose specified according to the body weight and were dissolved in normal saline. The 2000 mg/kg extracts were administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. The volume was maintained, not exceeding 1 ml/100-gram body weight. Normal saline was used as a vehicle, and the control group was fed with saline only.

The toxicity report data was prepared for the individual group and was summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility.

Results and Discussion

Phytochemical screening

The results of the various phytochemical screening tests obtained during the experiment are shown in the table below (Table 2). Flavonoids, alkaloids, steroids, glycosides etc. were the phytoconstituents found in plants. The result of phytochemical analysis are consistent with previous studies that have screened for phytochemicals in *Asparagus racemosus* (Begum et al., 2017), *Bergenia ciliate* (Bhandari et al., 2019), *Withania somnifera* (Arya

& Chauhan, 2019), *Mentha piperita* (Patil et al., 2016), *Ocimum tenuiflorum* (Srinivas Naik et al., 2015), *Salvia rosmarinus* (Kontogianni et al., 2013), *Cymbopogon citratus* (Gupta et al., 2019), *Senegalia catechu* (Rekha et al., 2023), *Phyllanthus emblica* (Sapkota et al., 2022).

Total phenolic content

Phenolic compounds are believed to account for a major portion of the antioxidant capacity in medicinal and aromatic plants. The total phenolic contents of each constituent of herbal tea were estimated using the Folin–Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the FCR in an alkaline medium and is a simple and rapid method. The total phenolic content (TPC) of the individual medicinal plant of formulated herbal tea was determined and tabulated (Table 3). The TPC value of *Phyllanthus emblica* was determined to be 140.5 mg GAE/g, which agrees with the previous work done by Sabir and his group who reported 115.2 mg GAE/g ingrown fruits(Sabir et al., 2015). According to a research team of Nepal, the TPC value of *Senegalia catechu* extract was 55.21±11.09 mg GAE/gm (Shrestha et al., 2021), however, the value revealed while measuring the TPC content (97.769 mg GAE/gm) of *Senegalia catechu* used in tea formulation was substantially higher when compared to the literature's data. The total phenolic content of dried lemongrass leaf extracts was determined to be 55.362 mg GAE/g, which agrees with the data of group research, which was 43.17±0.67 mg GAE/g (Adeyemo et al., 2018).

According to a study conducted by Ulewicz-Magulska & Wesolowski, (2019) Mentha extract has a gallic acid equivalent phenolic content of 41.3 mg/gm, which supports the data of our study, which is 32.744 mg GAE/gm. According to Hossain et al., (2012), the ethanol extracts yielded 108.78±2.77 mg/gm gallic acid equivalent phenolic content in *Asparagus racemosus*, which is comparable to the results of our investigation. According to Kumar et al., (2018), the phenolic content of Ashwagandha root ranged from 0.09 to 0.69 percent. However, our investigation revealed a slightly greater phenolic content, i.e., 8.061 mg GAE/g, which is the major reason to keep it in mood freshener tea. According to Zafar et al., (2019) the TPC content of *Bergenia ciliata* is 88.40±1.12 mg GAE/g, whereas our investigation found the TPC to be 140.452 mg GAE/g. The results of our investigation suggest that the total phenolic content of basil is 16.468 mg/g, which roughly matches the data provided by a study reports that the TPC contents of basil was 12.60±1.02 (Wangcharoen & Morasuk, 2007). Stanciu et al., (2017) found rosemary to have the greatest total phenolic content of 608.37 mg GAE/g, but our investigation found rosemary to have a TPC of 132.907 mg GAE/g.

Table 2: Phytochemicals present in herbal tea formulation

Aqueous Plant Extracts	Phytochemical Tests							
Pakhanbed (*Bergenia ciliata*)	+++	+	+	+	+	++	+	-
Aswagandha (*Withania somnifera*)	+	+	+	+	-	+++	+	+
Asparagus (*Asparagus racemosus*)	+++	-	-	+	-	+++	+	-
Mentha (*Mentha piperita*)	+	++	+	+	+	++	-	-
Tulsi (*Ocimum tenuiflorum*)	++	+	+	+	-	+	-	+++
Rosemary (*Salvia rosmarinus*)	+	+	+	+	+	+++	+	-
Lemongrass (*Cymbopogon citratus*)	-	-	+	+	-	+	-	-
Khayar (*Senegalia catechu*)	+	+	+	+	+	-	-	+
Amala (*Phyllanthus emblica*)	-	-	-	+++	+++	+	-	-

Note: + sign indicates the presence of respective metabolite and – sign indicates its absence; number of + signifies the degree of metabolite's intensity

Table 3: Total phenolic content of the plants used in herbal tea formulation

S.N.	Plants	TPC (mgGAE/gm)
1	Amala (*Phyllanthus emblica)*	140.50
2	Khayar bark (*Senegalia catechu*)	97.77
3	Lemongrass (*Cymbopogon citratus*)	55.36
4	Mentha (*Mentha piperita*)	32.74
5	Kurilo (*Asparagus racemosus*)	10.63
6	Aswagandha (*Withania somnifera*)	8.06
7	Pakhanbed (*Bergenia ciliata*)	140.45
8	Tulsi (*Ocimum tenuiflorum*)	16.47
9	Rosemary (*Salvia rosmarinus*)	132.91

Acute oral toxicity

All the mice that received 2000 g/kg of aqueous extract of each constituent of herbal tea did not show any toxic signs and abnormal behavioral changes post 24 hrs of treatment and for 14 days observation days. The clinical signs and symptoms were the primary observations among several other toxicity indicators that reveal the toxic effects of medications on essential bodily organs. Despite considerable behavioral changes in the treatment group within the first 24 hrs, no animals were found dead. There were no notable changes in body weight over the 14 day acute toxicity study period, and food and drink consumption were both normal, which indicates the nutrients that makeup formulated tea are inevitable for multiple physiological functions in the body.

Table 4: Acute oral toxicity of herbal tea formulation

S.N.	Sample name	Dose (mg/kg)	Remark	Result
1.	Pakhanbed extract	2000	Not dead	Non-toxic
2.	Aswagandha extract	2000	Not dead	Non-toxic
3.	Asparagus extract	2000	Not dead	Non-toxic
4.	Mentha extract	2000	Not dead	Non-toxic
5.	Tulsi extract	2000	Not dead	Non-toxic
6.	Rosemary extract	2000	Not dead	Non-toxic
7.	Lemongrass extract	2000	Not dead	Non-toxic
8.	Khayar extract	2000	Not dead	Non-toxic
9.	Amala extract	2000	Not dead	Non-toxic

Proximate analysis of herbal tea formulation

The proximate composition of the formulated herbal tea was assessed by the AOAC method. Table 5 indicates the concentration of moisture content, crude fat, crude protein, crude fiber, total ash, carbohydrate and energy in each formulation. It is found that NPRL-1 has significant protein (7.38%), carbohydrate (72.25%) and energy (335.00 Kcal/100 g) compared to the other two formulations.

The NPRL-1 containing asparagus root as the significant component backs the fact it contains carbohydrates in the highest amount. Asparagus is a nutritious and healthy vegetable with ascorbic acid, vitamin B6, folic acid, rutin, saponin and glutathione, among other nutrients (Sun et al., 2005). In the proximate analysis study conducted by Saini et al. (2016), the carbohydrate content of *A. racemosus* roots and root powder were found to be 5.58±0.66% and 48.54±0.37%, respectively, showing that the root powder of *A. racemosus* contains a high amount of carbohydrate. In addition, Studies have established the beneficial effect of *Phyllanthus embilica*, another predominant constituent of NPRL-1 on pregnant women for its high enrichment in dietary fiber, potassium, copper, manganese, and important vitamins including vitamin C, B5, folic acid, and B6 make this plant noteworthy. Particularly folic acid is essential for reducing nausea and controlling hormone levels during pregnancy (Sharma et al., 2015).The another constituents *Elettaria cardamomum* also called as queen of spices was selected for the formulation due to its potential for better development of offspring with notable benefits in motor reflex development and weaning age. The administration of *Elettaria cardamomum* has also found a noteworthy result on female mice. A study conducted by Abu-Taweel GM revealed the administration of cardamom to female mice improved neurotransmitter activity, memory, and other behavioral attitudes (Abu-Taweel, 2018).

Likewise, NPRL-2 was targeted as the mood freshener tea with *Salvia rosmarinus*, *Senegalia catechu* and *Cymbopogon citratus* as the major components. The phytoconstituents of the rosemary is profoundly enlisted for its cognitive and calmness property to the human brain (Rahbardar & Hosseinzadeh, 2020) while, the substantial citral content of the lemongrass will make a fresh ambience for the freshness synergistically with the catechu biomolecules. The citral content in the lemongrass is found to be 65-85% and reported to have soothing

effect on brain and prevent the negative symptom of depression(Agarwal et al., 2022; Wilson et al., 2010). These cofounders of the constituents in NPRL-2 make a compete recipe for the mood fresher tea with soothing effect. The proximate analysis further back supports the proposed parameters for mood freshener tea with energy composition of 312.32 Kcal/100g, carbohydrate (66.63%), protein (6.55%) and fiber (10.96%) which suggest that this tea can provide individual with sustained energy throughout the day, promoting a refreshing and invigorating experience.

The formulation NPRL-3 was found to have a low concentration of carbohydrates (56.60%) with comparatively more fiber (20.83%), generating sufficient energy (278.48 Kcal/100g), which is beneficial to a diabetic patient. This statement aligns with the assertion made by the Centre of Disease Control and Prevention (CDC) regarding the role of fiber in diabetes management (Centre of Disease Control and Prevention [CDC], 2022). The energy concentration of the formulation is sufficient to combat the energy crisis caused by antidiabetic drugs consumed by diabetic patients. The major component of the NPRL-3 was Pakhanbed (*Bergenia ciliata*), followed by Tulsi (*Ocimum tenuiflorum*). The Ayurvedic preparations have used *Bergenia* species down the centuries for several ailments as this plant possesses a wide range of polyphenols, flavonoids, and quinines (Koul et al., 2020). The stem contains a chemical called Bergeniac-glycoside, which is also used for preparing medicine for Cancer. And the rhizome of *Bergenia* is used in the treatment of dysentery, fever, and kidney diseases (Gurung & Pyakurel, 2017). In contrast, Tulsi incorporates a good amount of protein and fiber. Ziemichód et al., (2019) have shown that holy basil seeds have a fiber content of 45.9% and a protein content of 21.5%.

Conclusion

Medicinal plants have long been employed as traditional healers for several ailments, and they have been used to make several medications. Here, with this study, we can conclude that the three different types of herbal tea, i.e., NPRL-1, NPRL-2 and NPRL-3 are non-toxic and show an array of potency to treat multiple ailments. The abundance of nutritional factors revealed through the proximate analysis and the presence of a plethora of phytochemicals with a remarkable phenolic content gives a way to explore these Nepalese medicinal plants for the formulation of herbal tea targeting specific physiology in the future. This preliminary formulation of tea employing the herbs of Nepal with nutritional quantification and oral safety will act as evidence for exploring bioactivities of the development of other nutraceutical products in the future.

Author Contributions

D. P. B, P. Y, A. P and A. K. M. conceptualized the entire research. A. P. and P P. wrote the Introduction. D. P. B., P. Y., A. K. M. and S. S. wrote Methodology as well as A. K. M., A. P. and S. S. wrote Results and Discussion, Conclusion. P. Y., D. P. B., A. K. M. and A. P. reviewed and edited the manuscript. D. P. B. and P. Y. spervised the entire research work. All authors have read and agreed to the published version of manuscrpts.

Table 5: Proximate analysis of herbal tea formulation

S.N.	Analytical Parameters	Results (%)		
		NPRL-1	NPRL-2	NPRL-3
1.	Moisture content	5.44	8.97	7.87
2.	Crude fat	1.46	1.83	3.02
3.	Crude protein	7.38	6.55	5.91
4.	Crude fiber	9.75	10.96	20.83
5.	Total ash	3.71	5.06	5.76
6.	Carbohydrate	72.25	66.63	56.60
7.	Energy	335.00 Kcal/100g	312.32 Kcal/100g	278.48 Kcal/100g

Acknowledgements

The Department of Plant Resources and the Natural Products Research Laboratory are acknowledged for providing budget and all the necessary facilities to conduct this research. Special thanks to the staff of NPRL for their technical assistance.

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