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Innovative Herbal Green Tea Formulation using Holarrhena antidysenterica, Emblica officinalis, and Stevia: Nutritional and Phytochemical Analysis

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ABSTRACT

As growing consumer awareness regarding the positive health benefits of herbal tea. The popularity of herbal tea has grown up become consumers are becoming more health-conscious towards their diets and they are transforming themselves towards alternative natural source of beverages. Herbal tea from Stevia, Amla, and Holarrhena antidysenterica offer unique health benefits. Stevia is a healthier alternative to sugar and regulates blood sugar levels, making it beneficial for diabetes patients. Amla, rich in vitamin C and antioxidants, strengthens the immune system, improves skin health, and promotes digestion. Holarrhena antidysenterica is used for digestive issues, strengthens the gastrointestinal tract. These teas contribute to overall well-being. The objective of the research is to development of Herbal Tea formulization and study its Nutritional & Phytochemical constituents as well as minerals contstituents (metals) of developed herbal tea from Holarrhena antidysenterica, Emblica officinalis & Stevia. The proximate analysis of moisture, ash, fat, protein, and carbohydrates in developed herbal tea was conducted on both crude and serve size basis. Carbohydrates, such as simple sugars, oligosaccharides, and poly- saccharides, react with strong acid and heat to form furan derivatives that condense with phenol to form stable yellow-gold compounds. The fibre content was determined by enzymatic method by using alpha amylase, protease and amyloglucosidase. The Folin–Ciocalteu assay is used to assess the total phenolic content. The inorganic content (metals) was analysed by ICP MS. The HPLC system (Agilent 1100, USA) is used to evaluate vitamin C by using 3% metaphosphoric acid and 8% acetic acid. The total phenolic content was found to be 6.50 µg GAE/mg in the crude formulation and 0.23 µg GAE/mg on a serving size basis. Vitamin C content was 7.94% in the crude formulation and 0.29% per serving. The nutritional composition of the crude formulated herbal tea includes protein at 8.48%, fat at 0.2%, carbohydrates at 37.73%, fiber at 22.18%, and energy at 186.91 kcal/100g. On a serving size basis, these values are 0.29% protein, <0.05% fat, 1.17% carbohydrates, 0.61% fiber, and 5.84 kcal. This formulated herbal tea is a rich source of minerals, phytochemicals, and vitamin C. The combination of herbs used in its formulation not only enhances its color, flavor, and aroma but also provides various health benefits, making it widely accepted for its numerous therapeutic advantages.

Keywords: *Herbal Tea, Holarrhena, antidysenterica, Emblica officinalis, Stevia, Nutritional, Antioxidant, Phytochemical, HPLC, ICPMS, UV Vis Spectroscopy.*

1. INTRODUCTION

Herbs are rich source of antioxidant & polyphenol and it has a broad range of bioactive compounds (bio active predominant components of herb extract and beverages) such as phenolic acids, polyphenols, flavonoids, tannins, terpenoids and vitamins. Herbs are a widely distributed in nature and widespread in various group of plants, excluding vegetables and other plants that are consumed for macronutrients, with savoury or aromatic properties which are used for flavouring and garnishing the

food, and for fragrances and medicinal purpose. Herbs generally refers to the leafy green or flowering parts of a plant (either fresh or dried), while spices are usually dried and produced from other parts of the plant, including seeds, bark, roots and fruits. (Parul Namdev, 2015). Drinking has always meant much more than satisfying the thirst. Drinking can be a necessity, an indulgence, a comfort or a social activity. *Liquid Pleasures* is an engrossing the study of social history of drink in Britain from the late seventeenth century to the present, from first cup of tea, at breakfast to mid-morning tea, to an evening tea and a 'night-cap'. (Burnett, 1999). Tea is popular next to water and it is the cheapest beverage for humans consume. Drinking the beverage tea has been considered a health promoting habit since ancient times. The modern medicinal research is providing a scientific basis for this belief and support that tea provides various health benefits. (Khan, 2013). Herbal tea is commonly used as a beverage brewed from the leaves, seeds, fruits, flowers, seeds, roots & stem of plants species rather than the Camellia sinensis, which has been used for prevention of disease & health care for the worldwide. (J. Zhao, 2013). The philosophy behind is prevent in unessential suffering and living a long healthy life. Medical Science involves the employment of natural elements to eliminate the root cause of the disease by restoring balance at the same time create a healthy life-style to prevent the recurrence of imbalance. In Medical Science/Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The Ayurvedic literature Sarangdhar Samhita highlighted the concept of poly-herbalism to achieve greater therapeutic efficacy. In majority of traditional systems, diabetes is best managed by the herbs combination (Polyherbal) instead of single herb because of synergism and its less side effects. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. (Rukaiya Shirohiwala, 2022).

Holarrhena antidysenterica

Holarrhena antidysenterica (HA), a twining shrub belonging to the Apocynaceae family and it is found in tropical regions of Africa and over a large part of Asia including India. In Indian traditional system of medicine, HA has been used to treat 36 gastric ailments, for wound healing and also to improve glycaemic control. HA commonly known as kurci, kurchi or kutaj is being used from ancient time in the treatment of amoebiasis, chronic bronchitis, diabetes and locally for boils and ulcers. (Keshri Umashanker Pd, 2012). HA belongs to the family Apocynaceae, is commonly known as kudu in Marathi. Plant parts like Root, stem, bark and seeds of HA was used in many traditional systems of medical science including Ayurveda and Unani. (Kawale, 2019). The ethanolic extract of HA seed has anti hyperglycaemic activity at to 250 to 500mg/kg & 300 to 600mg/kg & as it lowers serum glucose level in diabetic albino rats and significantly increases glucose tolerance. It prevents weight loss in diabetic rats and corrects altered biochemical parameters e.g., serum cholesterol, triglyceride, aspartate transaminase, alanine transaminase, alkaline transferase, total protein, urea, creatinine and uric acid to near normal physiological range. (Keshri Umashanker Pd, 2012) (Aymen Owais Ghauri S. A., 2020).

Stevia

Stevia (Stevia rebaudiana Bertoni) is a nutrient rich plant which belong to asteraceae family. The leave of stevia contains diterpene, stevioside, glycosides A-F, steviolbioside, rebaudiosides and dulcoside which are responsible for its sweetness. Stevia is known as natural sweetener. Stevia herb has low in calorie value, its dry leaves possess roughly 40 times more sweetness than sugar rebaudioside-A, are found to be 300 times sweeter than sugar. Stevia contains natural antioxidant which helps in lowering blood pressure, cholesterol, and control diabetes. (Suresh V, 2018). Stevia, a perennial shrub of the Composite family, is cultivated in many regions across the world. It is popular for its sweetness, which is due to the presence of steviol glycosides, having 100–300 times sweetness than the sucrose. It has been used as a sugar substitute or natural sweetener in the food and drug industry. Due to its rich nutritional and phytochemical profile, stevia also provides beneficial effects against a plethora of health conditions. (Jamil Ahmad, 2020). tevia is the new emerging alternative source of calorie free sweetener having no carbohydrate and fat. It is 20 to 30 times sweeter than cane & beet sugar and highly nutritious, delicious, non-toxic and non-additive sugar. It can enhance the flavour, used in weight reduction, helpful for digestion, contain antioxidant, prevents dental caries and having antimicrobial and anti-plaque properties, which increases the mental alertness, increase energy levels but does not affect the blood sugar level, therefore key-source sweetener for diabetic world. (S.D. Singh, 2005). S. rebaudiana, a perennial plant native to Paraguay, has come to be cultivated around the world as a source of high-potency sweetener with no caloric value. Two main steviol diterpene glycosides, stevioside and rebaudioside A, that are present in high levels in Stevia leaves provide the sweet taste of the plant i.e., 150-450 times sweeter than sucrose to human taste buds. In addition to different glycosides, Stevia leaves contain many other compounds like flavonoids and fatty acids that provide together many diverse biological properties of the plant. Thanks to these components, Stevia products stimulate insulin production in diabetics, improve polycystic kidney disease, have chemotherapeutic action in cancer and possess powerful antibacterial, antioxidant and immune-modulating properties (Victoria Peteliuka, 2021).

Emblica officinalis

Emblica officinalis is a medicinal natural gift plant to human lives and to promote disease free healthy life. It is commonly known as Amla. It has therapeutic potential against deleterious diseases and widely distributed in tropical and subtropical areas. From the early time, it become a notable fruit because of its rich amount of vitamin C, polyphenols such as tannins, ellagic acid gallic acid, flavonoids like rutin and quercetin. Emblica officinalis is a natural, efficacious an antioxidant with

the richest natural source of Vitamin C (200 to 900 mg per 100 g of edible portion), (Kaushik Vilas Kulkarni, 2018).

Amla has been found to possess rich phytochemistry distributed in different sections of the plant (fruits, leaves, and roots). Polyphenols comprise the main group of secondary metabolites wherein several compounds belonging to phenolic acids, flavonoids, tannins, other phenolics and derivatives compounds have been reported in different studies. Phytochemical analysis revealed important bioactive chemical compounds such as tannins, alkaloids, polyphenols, gallic acid, ellagic acid, emblicanin A and B, phyllembein, quercetin, ascorbic acids, vitamins and minerals. The extracts of amla possess the potent of antimicrobial activities to counter different bacterial pathogens. Amla phytochemicals also possess antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, immune modulatory, hypolipedemic, memory enhancing, anticancer, antidiabetic, antidepressant, anti-ulcerogenic, insecticidal, larvicidal, and wound healing activities (Sandip Kumar Khurana, 2019) (Bhavesh C. Variya, 2016).

2. MATERIALS AND METHODS:

Chemical:

Holarrhena antidysenterica seeds was collected form the local market of Ranchi (Jharkhand). Identification& Authencity were done by CSIR: National Institute of Science Communication & Policy Research. (Authentication No: NIScPR/RHMD/Counslt/2023/4636-37-2). The seeds were washed & dried at 39°C for 4 days and make it fine powder form by the help of grinder. Ethanolic extract (by 90% ethanol) by using soxhlet apparatus. The solvent was evaporated at reduced temperature & extract were stored at 0-4°C. (Keshri Umashanker Pd, 2012), (S. Kumar, 2015). **Emblica officinalis** was collected from local market of Gurug (Haryanaa). Identification & Authencity were done by CSIR: National Institute of Science Communication & Policy Research. (Authentication No: NIScPR/RHMD/Counslt/2023/4636-37-1). The fruit were wash properly & remove its seed & make it into granular form, then dry by vacuum oven at 70°C (Madhu Agarwal, 2012) (Pao Kongsoontornkijkul, 2006). **Stevia** rebaudiana leaves was collected from local market of Gurug (Haryanaa). Identification & Authencity were done by CSIR: National Institute of Science Communication & Policy Research. (Authentication No: NIScPR/RHMD/Counslt/2023/4636-37-3), and after that wash and dry at 40 ± 2 ◦C for 7 h and make it granular form. (Roberto Lemus-Mondaca, 2015)

Sample Preparation:

Take 0.9 g of Stevia rebaudiana (dry granular leaves), 0.7 g dry granules emblica officinnalis, 0.15 g Holarrhena antidysenterica seed extract & add 0.15 g vacuum dry pineapple granules. & homogenized it properly. After that, use this 2 g dry herbal tea, add 150 ml water (as serving size) & boiled for 1 minute. After that filter it & analysis its Phytochemicals, Vitamin C, Sugar profile, & Safety Parameter as per FSSAI, Tea Regulation as per serving size & as a crude product.

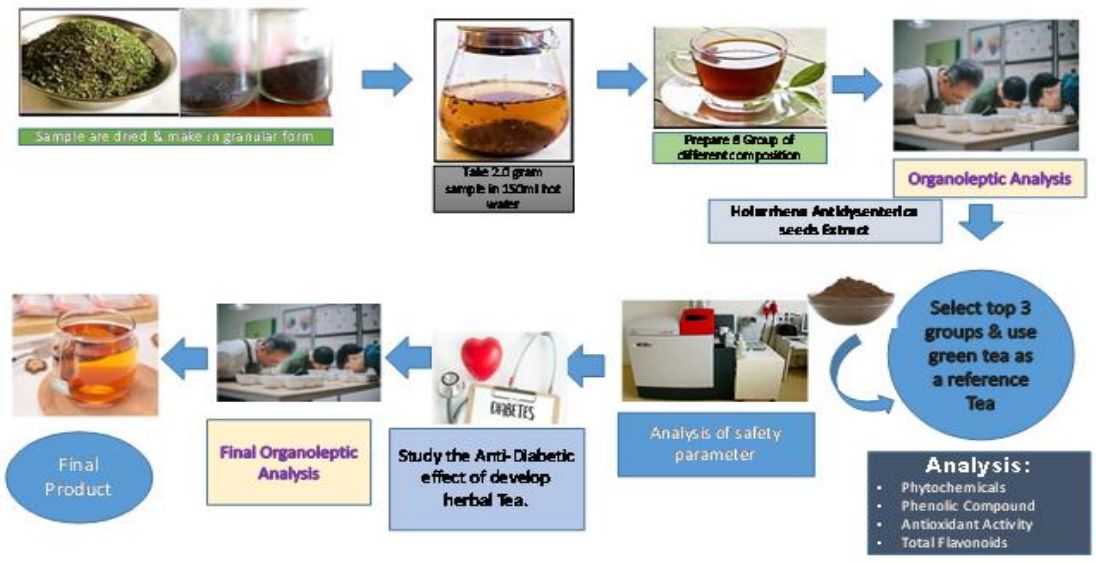


Fig: 1 Process Diag

Phytochemical Analysis:

The moisture content was determined by Hot Air oven method in which 2-g sample placed in a preheated dish and weighed and then dried in a hot air oven at 105 °C for 4 to 5 hrs or till constant weight reached. (M. S. Parvez, 2020) (Parul Namdev, 2015).

Ash:

Ash **content** was measured by taking 5 g of the sample and weighed accurately into a previously weighed crucible. The sample was burned at 550°C so that all the ingredients should burn except the minerals present in the foodstuff (M. S. Parvez, 2020).

Protein:

Protein content was determined by Kjeldahl technique (Parveen and Khatkar, 2015) in which sample used to estimate on the basis of protein levels. In this process Digestion, distillation, and titration are the main steps for determination of Protein in which sample was digested in concentrated sulphuric acid by using catalyst as copper sulphate & sodium sulphate. After digestion, 10 ml NaOH (40 % Sodium hydroxide) was added, followed by distillation. The distillate was then collected in a conical flask (volume 50 ml) containing 5 ml boric acid and 2 drops of indicator mixed until the solution changed color. The distillate was then titrated against standard hydrochloric acid to get the value. (M. S. Parvez, 2020).

Fat:

Fat was determined by using soxhlet extraction method was used to examine crude fat. In which 5 gs of sample was taken in pre-weighed thimbles. Extraction was performed for 15 to 18 hours using petroleum ether. (M. S. Parvez, 2020)

Vitamin C:

2.5 g of ground dried sample was transferred to 25 ml volumetric flask, 3% metaphosporic acid in 8% acetic acid was added and the mass was mixed for 5 min. The flask was filled up to the volume and filtered. Activated carbon was added to the filtered solution to remove the colour and filtered through filter paper (blue label) and membrane syringe filter with diameter pore of 0.45 µm. The filtrate was used for HPLC analysis of vitamin C at the HPLC system (Agilent 1100, USA) equipped with C-8 column and DAD detector. Mobile phase (0.1 M ammonia-acetate) flow rate was 0.4 ml/min and column temperature 37◦C. (Senka Vidovic, 2013)

Determination of Total Phenolics:

Total phenolics were determined using the Folin–Ciocalteu assay. (Sharifah Soplah Syed Abdullah, 2020). The absorbance was measured at 765 nm and the results were expressed in gallic acid equivalents. (Parul Namdev, 2015). Total phenolic content was determined according to a modified procedure from Singleton *et al*. (1999). The extracts of the herbal tea formulas (100 µL) were oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na2CO3. The absorbance was measured at 765 nm by a UV-Vis spectrophotometer after being mixed and incubated in room temperature for 30 min. The results were expressed as gallic acid equivalent (mgGE/gExt). (Rachanee Nammatra, 2021). Phenolic content compounds in investigated samples were extracted using methanol as an extraction solvent and were determined using standard Folin–Ciocalteu spectrophotometric procedure (Singletonand Rossi, 1965; Kähkönen et al., 1999). Before the extraction investigated samples were ground in a blender. 5.0 g of this way prepared sample was transferred to the volumetric flask after 50 ml of extraction solvent was added. The samples were shaken (GFL, Schuttel apparate Shakers, Germany, Model 3015) in the darkness for 24 h, at room temperature, and filtered. Immediately after filtration the content of total phenolic compounds was determined by Folin–Ciocalteu procedure by using galic acid as a standard compound. Absorbance was measured at 765 nm. Content of total phenolic compounds has been expressed as mg of gallic acid equivalent per 1 g investigated sample (mg GAE/1 g). All analysis were performed in three replicates (Senka Vidovic, 2013).

Carbohydrate:

Total carbohydrate was determined by Phenol Sulphuric Acid Method: In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. It forms a green coloured product with phenol and shows absorption maximum at 490nm. Weigh about 100 mg of the sample into a boiling tube. Hydrolyse it by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCL and cool to room temperature. Neutralize it with solid sodium carbonate till the effervescence ceases. Make up volume up to 100 ml and then centrifuge it. Pipette out 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard into a series of test tubes. Pipette out 0.1 and 0.2 ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1 ml with water. Set a blank with 1ml water. Add 1 ml of phenol solution to each tube. Add 5 ml of 96% sulphuric acid to each tube and shake well. After 10 min shake the contents in the tubes and place in water bath at 25-30°C for 20 min. Read the colour at 490 nm. (G.Gurunani, 2021)

Safety Parameter as Heavy metals:

About 200 mg of herbal samples were accurately weighed into a PTFE digestion vessel. 5 mL of concentrated HNO3 and 3 mL of concentrated H2O2 were added to the vessel and waited for about 20 min before the vessel is closed. Decomposition of the samples was carried out in a microwave digestion system (Bergh of, Germany, 2008). A two-step progme (Table 1) was applied to the samples. The undissolved parts were separated with centrifugation at 4000 rpm for 10 min. The extract was transferred into a volumetric flask and made up to 25 mL with double distilled water. Blank experiments (n = 3) were carried out in the same way. The certified reference material analysis (GBW07605 tea sample) was made by using dissolving method mentioned above. (Tokalıoglu, 2012) (Angela Giorgia Potorti, 2020)

3. RESULTS AND DISCUSSION

Proximate analysis:

Table 1 showed the results obtained for the proximate analysis of Moisture, Ash, Fat, Protein, and Carbohydrates & Energy in Developed Herbal Tea on Crude Basis as well as on Serve Size Basis.

Carbohydrates (simple sugars, oligosaccharides, poly- saccharides, and their derivatives) react in the presence of strong acid and heat to generate furan derivatives that condense with phenol to form stable yellow-gold compounds that can be measured spectrophotometric at 490 nm. Read the standard curve tubes from low to high concentration (i.e., 20 µg/2 mL up to 100 µg/2 mL), and then read your beverage samples. To be sure that the outside of the cuvettes is free of moisture and smudges, wipe the outside of the cuvette with a clean paper wipe prior to inserting it into the spectrophotometer for a reading.

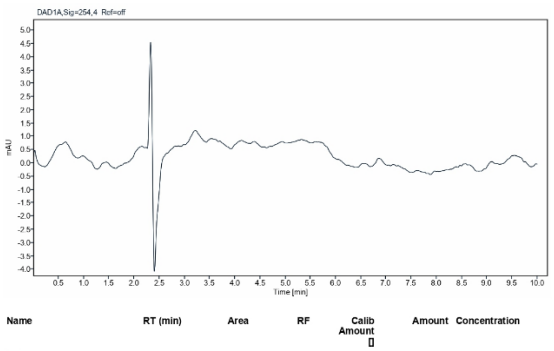
Dietary Fibre Duplicate test portions of dried foods, fat-extracted if containing >10% fat, are gelatinized with Termamyl (heat-stable a-amylase), and then enzymatically digested with protease and amyloglucosidase to re move protein and starch. (When analyzing mixed diets, always extract fat prior to determining total dietary fibre.) Four volumes of ethyl alcohol are added to precipitate soluble dietary fibre. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and ac e tone. After drying, residue is weighed. One duplicate is analysed for protein, and other is incinerated at 525°C and ash is determined. Total dietary fibre = weight residue – weight (protein + ash).

Parameter	Crude Basis	Serving Size Basis
Moisture (%)	6.77	98.34
Ash (%)	6.58	0.21
Fat (%)	0.23	<0.05
Protein (%)	8.48	0.29
Carbohydrates (%)	37.73	1.17
Energy (Kcal/100g)	186.91	5.84
Dietary Fibre (%)	22.18	0.61

Table 1. Proximate Analysis on Crude Basis as well as on Serve Size Basis.

Phytochemical characteristics

The result of Vitamin C analysis in Developed Herbal Tea on Crude Basis as well as on Serve Size Basis was mentioned in Table 2. Vitamin C content in fresh amla was 533 mg/100gm which was observed by Hasan *et al.* (2016).



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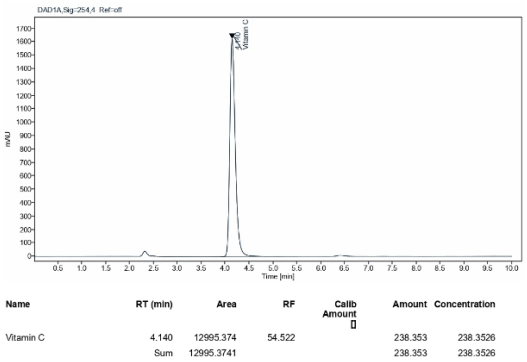

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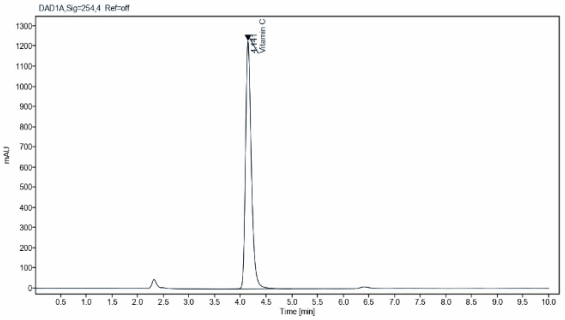
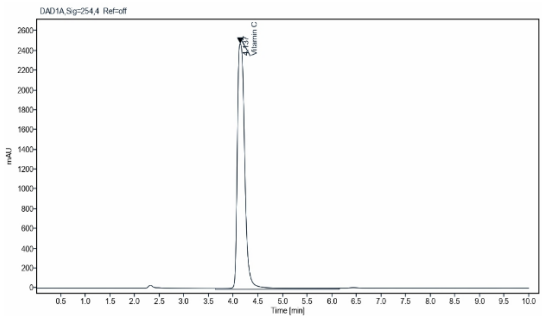


Name	RT (min)	Area	RF	Calib Amount	Amount	Concentration
Vitamin C	4.141	9695.750	54.820		176.865	176.8646
Sum		9695.7497			176.865	176.8646

On serve Basis



Name	RT (min)	Area	RF	Calib Amount	Amount	Concentration
Vitamin C	4.137	26914.514	54.074	500.000	497.734	497.7336
Sum		26914.5145			497.734	497.7336

on Crude Basis

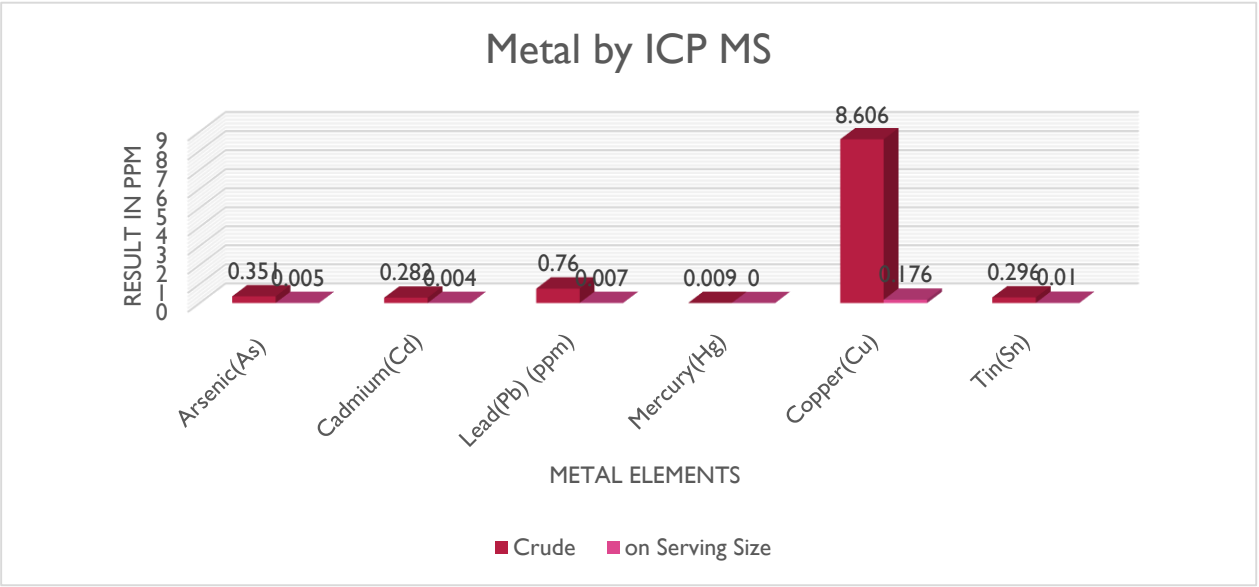
Graph: 1 Vitamin C

The total phenolic contents (TPC) were estimated using gallic acid standard. The TPC analyses in Developed Herbal Tea on Crude Basis as well as on Serve Size Basis was mentioned in Table 2. Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants with variable properties, and are thought to have positive effects on human health.

Parameter	Crude Basis	Serving Size Basis
Vitamin C(g/100g)	7.94	0.29
Phenolic Compound GAE equivalents (µg GAE/mg sample)	6.5	0.23

Table 2. Vitamin & phenolic content on Crude Basis as well as on Serve Size Basis.

The concentrations of Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg), Copper (Cu) & Tin (Sn) in Developed Herbal Tea on Crude Basis as well as on Serve Size Basis was mentioned in Table 3 which were determined by using ICP- MS. The results indicated that the concentrations of elements determined by ICP- MS method are in agreement with the certified values (Table 3).



Graph: 2 Metal Elements

Metals	**Crude Basis**(ppm)	**Serving Size Basis**(ppm)
Arsenic (As)	0.351	0.005
Cadmium (Cd) (ppm)	0.282	0.004
Lead (Pb) (ppm)	0.760	0.007
Mercury (Hg) (ppm)	0.009	0.00
Copper (Cu) (ppm)	8.606	0.176
Tin (Sn) (ppm)	0.296	0.01

Table 3. Metals Analysis on Crude Basis and Serve Size Basis.

4. CONCLUSION

According to the findings obtained from the nutritional, phytochemical and antioxidant activity of developed herbal tea from Holarrhena antidysenterica, Emblica officinalis & Stevia, the potential to be an immense source of flavouring agents and nutraceuticals. The amalgamated formulations have many positive health effects make it the ideal physical and mental wellness revitalizing agents. The creation of herbal teas intended target specific health benefits like gut health, anxiety relaxation, and controlling obesity. These regular drinks possessed high levels of antioxidants and total phenolics. Such drinks may be significant dietary supplements sources of phenolics, which are antioxidants that help inhibit oxidative stress-related illnesses. The findings of this research provided consumers, dietitians, and food regulatory bodies with newly acquired understanding on these beverages' antioxidant function. The epidemiologic method should be used in further studies to explore the adverse medicinal effects of these drinks on consumers being produced using herbs, enhancing its appearance, taste, aroma, and widespread acceptability along with several categories of nutritional as well as medicinal advantage.

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