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Preparation And Evaluation of Herbal Tea Powder

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Abstract

Tea is a prevalent and focal point for cultural and social gathering. It is a preparation which boosts up immunity, keeps active, rejuvenates cells it relieves stress, fatigueness, tiredness and anxiety. The aim of present study is to prepare herbal tea with new combination of medicinal plants i.e. star anise, tulsi, black pepper, amla, stevia, lemon grass with the possibility to have maximum therapeutic benefits and suitable consumption. The medicinal plants selected here are reported for various activities such as anti-influenza, immunostimulant, anti- bacterial, bioavailability enhancer, vitamin C supplement, sweetner, flavorant and colorant respectively. The decoction of tea powder containing the above medicinal plants is evaluated for qualitative and quantitative estimations for carbohydrate, ascorbic acid, protein, tannins, and phenolic acid. The antioxidant activity has also been performed.

Keywords

Herbal tea, immunity, antioxidant, anti-anxiety.

INTRODUCTION:

Tea is a prevalent and focal point of cultural and social gathering. Tea is the most generally consumed beverage after water. It has cooling, slightly bitter, and astringent flavor that many people enjoy. Tea is one of the most popular beverages, consumed daily in all domestic, social and official meeting. It is a preparation which boosts up immunity, keeps active, rejuvenates cells, relieves stress, fatigueness, tiredness, anxiety and many more[1].

British introduced tea into India in an attempt to break the Chinese monopoly on tea [2]. Herbal tea or tisane is any beverage made with the infusion or decoction of herbs, spices, or other plant material in hot water, and usually does not contain caffeine[3]. These drinks are distinguished from caffeinated true teas which are prepared from the cured leaves of the tea plant, Camellia sinensis, as well as from decaffeinated tea, in which the caffeine has been removed. In addition to serving as a beverage, many herbal teas are also consumed for their apparent medicinal benefits [4]. Herbal tea is in fact a catch all term used for any non-caffeinated beverages made from the infusion or decoction of herbs, spices, or other plant material, hence in some countries like in Europe, tisanes or herbal teas are also known as infusions.

Herbal Tea Varieties:

Depending on the plant used and on the method of preparation the beverage, there are many varieties of herbal tea. Many more herbal tea varieties can be found than tea varieties for one simple reason: tea is extracted from one plant, tisane is made from many. *Anise tea* is well-liked in the Mediterranean region and in the Southwest Asia where the anise plant is native. It is sweet and highly aromatic, notable by its characteristic flavor.

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Artichoke tea made from the artichoke plant indigenous to the Mediterranean region in Europe. It has a slightly bitter and woody taste.

Burdock tea made from the plant also known as arctium, a plant indigenous to Europe. It features a pungent flavor with a little muddy harshness. It is not recommended to pregnant women because it is known to stimulate the uterus.

Chamomile tea is most frequently used as a sedative. Chamomile flower can be found mostly in temperate areas throughout Europe and Asia [5].

Herbal tea:

The term herbal tea is really a misnomer, as herbal teas don't contain any tea leaves. All teas harvested from the tea plant will contain caffeine. It is a natural part of the plant. Herbal "teas" does not contain caffeine, and that is because they don't actually contain tea! The appropriate name for an herbal tea is 'Tisane.' Herbal Tisanes ("teas") are made up of various flowers, herbs, spices, and dried fruits which are naturally caffeine free; such as chamomile flowers, lemongrass, basil, rose buds,etc. Often times, herbs, spices, or fruits will be added to an actual tea for flavoring. If there is no tea listed in the herbal blend, then there is no caffeine, but if tea leaves is one of the ingredients, it will contain caffeine [6].

Health benefits of herbal tea:

Tisanes are most of the time consumed due to their physical or medicinal property, especially for their stimulant, relaxant or sedative properties.

Tisanes are available pure or in mixture with other ingredients featuring thus different antioxidant properties and therapeutic applications depending on the variety.

The antioxidant properties of tisanes from moderate regions have been well-studied while those originating in tropical regions are still an unknown territory, yet to be uncovered. There are countries as Unites States for example, where marketers of tisanes are not allowed to state unconfirmed claims on the beverage's wonder effects when health is concerned [5].

MATERIAL AND METHODS: -

The plants containing antioxidant property were selected according to their medicinal uses from various categories like anti-bacterial, anti-influenza, anti-diabetic, anti-obesity. The crude drugs like Turmeric, Tulsi, Stevia, Long pepper, Lemongrass, Amla and Star anise to be used to prepare herbal tea was collected from local market and were authenticated from Piramal Life Sciences. The formulation will be beneficial for every individual to develop immune system.

Chemicals used are of Analytical reagent (AR) grade (LOBA Chemicals Ltd.).

The in-house herbal tea was prepared by the three different combinations as follows.

| Sr.no | Ingredient | F2A (g) | F2B (g) | F2C (g) |
|-------|-------------|---------|---------|---------|
| 1 | Star anise | 1 | 1 | 1 |
| 2 | Tulsi | 1 | 1 | 1 |
| 3 | Long pepper | 1 | 2 | 1 |
| 4 | Lemon grass | 1 | 1 | - |
| 5 | Amla | 1 | 1 | - |
| 6 | Stevia | 1 | 1 | 1 |
| 7 | Turmeric | 1 | 1 | 1 |

Table no 1: Composition of in-house Herbal tea formulation

Preparation of Medicated Herbal Tea in Laboratory:

The materials were shade dried and reduced to coarse powder. The powder was passed through appropriate sieve and was weighed accurately. The F2A, F2B and F2C were formulated as per the table no. 1.

Approximately 2g powder was packed in a single tea bag.

EVALUATIONS

Physicochemical Evaluation:

Organoleptic evaluation ^[7]: Morphological evaluation such as color, odour and taste were carried out.

Ash value ^{[8][9]}: Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'Total Ash Value'. Such variables are then removed by treating with acid as they are soluble in HCL acid and then acid insoluble and water-soluble ash value is determined.



Determination of Total Ash Value:

Weigh accurately 2g of the air-dried drug in a tarred silica crucible and incinerate at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon-free ash is not obtained, wash the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly white, add the filtrate to the dish, evaporate to dryness and ignite at a temperature not exceeding 450º. Calculate the percentage of total ash on the dried drug basis.

Determination of Acid Insoluble Ash Value: Boil the ash with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, ignite, cool in a desiccators and weigh. Calculate the percentage of acid-insoluble ash on the dried basis.

Determination of Water-Soluble Ash Value: Boil the ash for 5 minutes with 25 ml of water, collect the insoluble matter in a Gooch crucible or an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Calculate the weight of the insoluble matter subtracting from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the water-soluble ash value on the dried basis.

Extractive value [8][9] : Take accurately weighed quantity of sample and macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during 6 hours and allowing standing for 18 hours. Filter the extract and avoid the loss of solvent. 25 ml of the filtrate was taken and evaporated to dryness in a tarred crucible at 105°C, to constant weight and weighed the percentage of water-soluble extractive was calculated.

Loss on drying [8][9]: Loss on drying is the loss of weight expressed as % w/w resulting from water and volatile matter can be driven off under specified conditions. Weigh about 2 gm of the air-dried crude drug in a dried and tarred flat weighing dish. Dry in oven at 100-105°C. Cool in desiccators over phosphorus pentaoxide for specific period of time. The loss in weight is recorded as moisture. Repeat the process till constant weight is obtained.

Qualitative estimation [10]: The decoction of marketed tea was subjected to phytochemical screening for identification of different phytoconstituents like carbohydrate, tannins, alkaloid, proteins and flavonoids.

Quantitative estimation ^{[11][12][13]}:

Estimation of Total Carbohydrate by Phenol Sulphuric Acid Method: In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. It forms a green colored product with phenol and shows absorption maximum at 490nm. Weigh about 100 mg of the sample into a boiling tube. Hydrolyze 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 color at 490 nm.

Estimation of Proteins by Bradford Method: The assay is based on the ability of proteins to bind Coomassie brilliant blue G 250 and form a complex whose extinction coefficient is much greater than that of the free dye. Prepare a series of protein samples in test tubes in the concentration. This is prepared in PBS. preferably Prepare the experimental samples in 100 μ l of PBS. Add 5 ml of diluted dye binding solution to each tube. Mix well and allow the color to develop for at least 5 min but no longer than 30 min. The red dye turns to blue when it binds protein. Plot a standard curve using the standard protein absorbance V concentration. Calculate the protein in the experimental sample using the standard curve.

Estimation of Total Free Amino Acids: Ninhydrin, a powerful oxidizing agent, decarboxylates the alphaamino acids and yields intensely coloured bluish purple product which is calorimetrically measured at 570 nm.

Ninhydrin + Alpha-amino acid - Hydrindantin +Decarboxylated amino acid + Carbon dioxide + Ammonia

Hydrindantin + Ninhydrin + Ammonia Purple coloured product + Water

To 0.1 ml of extract, add 1 ml of ninhydrin solution.Make up the volume to 2 ml with distilled water.Heat the tube in a boiling water bath for 20 min.Add 5 ml of the diluent and mix the contents.After 15 min read the intensity of the purple color against a reagent blank in colorimeter at 570 nm. The color is stable for 1 hr. Prepare the reagent blank as above by taking 0.1 ml of 80% ethanol instead of the extract.

Estimation of total tannins content by Folin-Denis like method: Tannin compounds reduces phosphotungstomolybdic acid in alkaline solution to



produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins. Transfer 1 ml of the sample extract to a 100 ml volumetric flask containing 75 ml water. Add 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution and dilute to 100 ml with water. Shake well. Read the absorbance at 700 nm after 30 min. Prepare a blank with water instead of the sample. Prepare a standard graph by using 0-100 µg tannic acid.

Estimation of Total Flavonoid Content: Take 1 ml sample of aqueous extract of tea powder. To each 10ml of analyzed solution, 2ml of water and 5ml of AlCl₃ reagent was added (133mg crystalline aluminum chloride and 400mg crystalline sodium acetate were dissolved in 100ml of extracting solvent) and absorbance recorded at 430nm against the blank (10ml of analyzed solution + 5ml of water). The amt of flavonoids was calculated as a quercetin equivalent from the calibration curve of quercetin standard solution and expressed an mg quercetin/ 100gm of extract.

Estimation of Total Phenolic Content: Phenols react with phosphomolybdic acid in Folin Ciocaltue reagent in alkaline medium and produce blue color complex (molybdenum blue).

Weigh exactly 0.5-1.0 g of the sample and grind it with a pestle and mortar in 10-time volume of 80%

ethanol. Centrifuge the homogenate at 10,000 rpm for 20 min. save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5 ml). Pipette out different aliquots (0.2-2 ml) into test tubes. Make up the volume in each tube to 3 ml with water. Add 0.5 ml oh Folin- Ciocaltue Reagent. After 3 min, add 2 ml of 20% Na₂CO₃ solution to each tube. Mix thoroughly. Place the tubes in a boiling water for exactly one min, cool and measure the absorbance at 650 nm against blank. Prepare a standard curve using different concentrations of Catechol.

Estimation of *In-vitro* Antioxidant Activity: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method:

Extract or standard solution 1ml (10-100 μ g/ml) was added to 2ml of DPPH in methanol (100mM). After incubation at 37°C for 30 min, the final volume was made up to 4 ml with methanol and the absorbance of reaction mixture was determined at 517 nm using spectrophotometer. The corresponding blank readings were also taken and the known radical scavenger, ascorbic acid was used as a positive control. Percent inhibition was calculated by comparing the absorbance values of control and test extract.

| luation: | | | | | |
|-------------|----------------|----------------|----------------|--|--|
| Evaluations | F1 | F2 | F3 | | |
| Color | Brownish green | Brownish green | Brownish green | | |
| Odor | Aromatic | Aromatic | Aromatic | | |
| Taste | Astringent, | Astringent, | Astringent | | |
| | sour, | sour, | , pungent, | | |
| | sweet | pungent, | sweet | | |
| | | sweet | | | |

RESULT AND DISCUSSION: Morphological Evaluation:

Table no 2: Morphological evaluation

Morphological evaluation of powder was done. The decoction of each tea was tasted. It was found that F2B was most acceptable than other two formulation

as it possesses a unique sour, sweet and pungent taste.

Physicochemical Evaluation:

| Parameter | F1 | F2 | F3 |
|---|--------------|--------------|--------------|
| Total ash | 7.2031±0.01 | 8.18±0.03 | 7.50±0.29 |
| Acid insoluble ash | 0.3202±0.02 | 0.3675±0.11 | 0.4628±0.01 |
| Water soluble ash | 4.555±0.43 | 4.775±0.18 | 4.767±0.18 |
| Extractive value as per IP | 21.3224±0.46 | 21.698±0.61 | 18.0933±0.46 |
| 5 min boiling extractive value | 13.4433±0.56 | 13.64±0.47 | 12.0745±0.05 |
| extractive value after dipping tea bag in hot water for 5 min | 13.3224±0.46 | 12.698±0.61 | 12.0933±0.46 |
| Loss on drying | 4.35±0.55 | 4.32±0.15 | 4.29±0.59 |
| Bulk density | 0.3251±0.01 | 0.3224±0.01 | 0.3255±0.01 |
| Tapped density | 0.3372±0.01 | 0.3365±0.01 | 0.3269±0.01 |
| Angle of repose | 40.651±0.66 | 40.4613±0.52 | 40.2246±0.60 |



| Carr's index | 5.5166±0.17 | 5.7033±0.27 | 5.4966±0.13 |
|----------------|--------------------------------|-------------|-------------|
| Housner'sRatio | 1.0653±0.01 (Mean ±SD, n=3) | 1.0647±0.01 | 1.0667±0.01 |

Table no 3: Physicochemical Evaluation

The physicochemical evaluation of all the three herbal tea formulations were done. The total ash value was found to be more in F2 formulation (8.18±0.03) whereas the acid insoluble was found to be more in F3(0.4628±0.01) and water-soluble ash was found to be more in F2(4.775±0.18). The extractive value was found to be more in F2 for extractive value as per IP, 5 min boling extractive value (13.64±0.47) and for 5 min Deeping in hot

water (21.698±0.61). The loss on drying was found to be more in F1 formulations (4.35±0.55). The bulk density was found to be more in F3 (0.3255±0.01), tapped density was more in F2 (0.3372±0.01), angle of repose was found to be more in F2(40.651±0.66), Carr's index was found to be more in F2 (5.7033±0.27), Haunsner's Ratio was found to be more in F3(1.0667±0.01).

Phytochemical Evaluation: -

| Chemical test | F1 | | F2 | F3 |
|------------------------------|----|---|----|----|
| Carbohydrate | | | | |
| Molisch test | + | | + | + |
| Fehling's test | + | | + | + |
| Benedict's test | + | | + | + |
| Bradford test | + | | + | + |
| Proteins | | | | |
| Biuret test | + | | + | + |
| Millions test | + | | + | + |
| Flavonoids | | | | |
| Shinoda test | | _ | _ | _ |
| Tannins and phenolic content | | | | |
| 5% ferric chloride solution | + | | + | + |
| Lead acetate test | + | | + | + |
| Bromine water test | _ | | _ | _ |
| Acetic acid | _ | | _ | _ |
| Potassium dichromate | _ | | _ | _ |
| Dil.nitric acid | + | | + | + |
| Dil. Iodine solution | _ | | _ | _ |
| Alkaloids | | | | |
| Dragandorff's test | _ | | _ | _ |
| Mayer's test | _ | | _ | _ |
| Hager's test | _ | | _ | _ |
| Wagner's test | _ | | _ | _ |
| Steroids | · | | - | |
| Salkowski test | _ | | _ | _ |
| Libermann- Burchard test | _ | | _ | _ |

Table no 4: Phytochemical Evaluation

The phytochemical evaluation of aqueous extract of all the three in house herbal tea were carried out in which the carbohydrates, proteins, amino acids, tannins were found to be positive.

CHROMATOGRAPHIC EVALUATION:

Number of solvent systems were tried for the chromatographic evaluation of F2 and F3 formulation. Solvent system which is reported for tannins shows spots in both F2 and F3.

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| Sr. no | Solvent system | | f spots | Detecting agent |
|--------|--|-----|---------|-----------------|
| | | F2B | F2C | - |
| 1. | A-Ethyl acetate: methanol: water | - | - | Iodine vapour |
| | 5:3:2 | | | |
| 2. | B-Ethyl acetate: Acetic Acid: Formic Acid: Water 75:2: 3: 20 | 5 | 4 | Iodine vapour |

Table no 5: Thin Layer Chromatography

QUANTITATIVE ESTIMATIONS OF F1, F2 and F3 FORMULATION:

| Sr.no | Estimation | F1 | F2 | F3 | |
|-----------------|---------------------|--------------|--------------|--------------|--|
| 1 | Carbohydrate | 0.2851±0.02 | 0.3141±0.01 | 0.3086±0.01 | |
| 2 | Proteins | 41.5233±0.57 | 44.8333±0.56 | 45.11±0.10 | |
| 3 | Amino acid | 0.4086±0.01 | 0.4113±0.01 | 0.4123±0.01 | |
| 4 | Ascorbic acid | 10.19±0.32 | 11.9726±0.38 | 1.0515±0.15 | |
| 5 | Total tannins | 0.0575±0.01 | 0.0579±0.01 | 0.0542±0.01 | |
| 6 | Total flavonoids | 11.706±0.04 | 13.636±0.12 | 11.6916±0.02 | |
| 7 | Total phenolic | 29.058±0.07 | 30.7946±0.06 | 28.7783±0.08 | |
| 8 | Antioxidant by DPPH | 45.7033±0.06 | 47.3133±0.03 | 42.9033±0.01 | |
| (Mean ±SD, n=3) | | | | | |

Table no 6: Quantitative Estimations of F1, F2 and F3 formulation

The quantitative estimations of all the three in house herbal tea were performed and reported. The total carbohydrate content, protein content, amino acid content, total tannins content, total phenolic content, total flavonoids content and antioxidant content were found to be more in F2 formulation.

CONCLUSION:

Consumption of tea as a beverage, health drink or medicated tea needs to be promoted for research and its publication. The detailed literature survey was done, and it was found that the tea can be a interesting topic of research. Here a new combination of herbal tea has been prepared by using the plant material like turmeric and amla which were collected from local area and star anise, long pepper, tulsi, lemongrass, stevia and the evaluation was performed by studying its morphological, physicochemical, phytochemical, and quantitative parameters. To prepare an ideal herbal tea we have selected the above herbs which have various beneficial uses like anti-inflammatory agent, antiflu, vitamin C supplement (ascorbic acid), а bioavailability enhancer, antimicrobial agent, natural sweetner, flavorant. The herbal tea with above formulation has less side effects and it is caffeine free so it does not causes addiction. From the above data we conclude that the F2 batch has maximum antioxidant, polyphenolic and tannins content. This formulation was also appreciated in terms of flavour by majority of people. So we can conclude that F2 formulation serves as best herbal tea than any other because this tea provides tea various phytoconstituents which we never include in our

daily diet. This tea can be served as ideal tea for diabetic, obese and people complaining digestive upset.So we can conclude that this tea can help in maintaining the immunity for healthy lifestyle with a cup of tea.

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