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Formulation and evaluation of polyherbal tea granules

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Abstract

The objective of the present study was to formulate and evaluate polyherbal tea granules for their physicochemical properties, stability, and antioxidant activity. The polyherbal tea granules were prepared by the wet granulation method and contained starch (rice broth) and natural plant leaves of moringa, tea, giloy, bay leaves, clove oil, and cardamom. The tea granules were subjected to phytochemical screening, quantitative phytoconstituent analysis, and several evaluation parameters like tapped density, bulk density, Hauser's ratio, Carr's index, disintegration, and moisture content. All formulations (F1, F2, F3, F4, and F5) are acceptable, but F2 is best in phytochemical screening according to the F1, F3, F4, and F5 formulations. The result of all evaluation parameters was evaluated to an acceptable limit, and a quantitative test of herbal tea granules is going on. The current formulation reveals an antioxidant response by the oral route of administration in water. Formulation to be the supplement of tea simultaneously stimulation response.

KEY WORDS: herbal tea, antioxidant activity, therapeutics uses, moringa, giloy, cardamom, tea, clove-oil and bay leave etc.

1. INTRODUCTION

Tea is the second most commonly consumed liquid refreshment after water. It is very useful for our health. It has many flavours, so people enjoy it very much. Nowadays, tea is popular everywhere and is consumed daily in social life. Tea helps relieve stress and fatigue in the immune system, etc[1].

Herbal tea is known as a polyherbal drink with medicinal value and was previously known as tisanes. Tisanes are herbal teas made from a mixture or combination of bark spices, plant flowers, fragrant herbs, seeds, leaves, grasses, etc., which give them their flavour and taste[2].

Herbal tea is modulated from tea leaves, seeds, flowers, etc. from plant species, including *Camilla sinensis* L.[3]. At present, as people are getting to know about the benefits of herbal tea, it is taking herbal tea to a different level. It has many medical benefits, and every day new research is being done for more therapeutic use, and herbal tea is available in almost every variety on the market[4,5]. The plant was washed thoroughly under running water to remove contamination and was shade dried with active ventilation at ambient temperature for 5 days; the dried aerial parts were ground to a fine powder using pistil and mortar. In today's time, tea is made from herbs, so it is gaining in popularity as consumers believe it is safe and may help boost digestion[6].

1.1 Antioxidant

The ability of tea content to prevent or postpone the oxidation of other molecules in food and biological systems is known as antioxidants[7,8]. Antioxidants have been investigated for the prevention of diseases like sickness of altitude, cancer, and heart disease (coronary disease). According to recent studies, antioxidant supplements are suggested to improve health in two types, as follows[9].

- Hydrophobic antioxidants are soluble in lipids. They protect cell membranes against lipid peroxidation.
- Hydrophilic antioxidants are soluble in water. They react with oxidants in the blood plasma and the cell cytoplasm[10].

2. MATERIALS AND METHODS

The current study's research was done at the HR Institute of

Pharmacy, Ghaziabad. Tea leaves were purchased from Golden Tips, the world's finest tea company, since 1933, and herbal plants were selected based on their health benefits. All herbal plants, like moringa leaves, cardamom, bay leaves, and giloy, were collected from nearby plants. and clove oil was purchased from the local retailer in Khora Market, Ghaziabad.

2.1 Sample preparation and blending process

Moringa leaves (drumstick), tea leaves, bay leaf, giloy leaves, and cardamom fruit were inspected manually to remove the yellow, dead, diseased leaf. Leaves were washed thoroughly to remove foreign materials and dried. All dried leaves were separately powdered with the help of a mixer grinder.

Table 1: Various plants used for herb tea

S.no.	Plant parts	Plant source
1.	Bark Tisanes	Cinnamon, black cherry bark
2.	Flower petals Tisanes	Hibiscus, Lavender, Rose, Chamoline
3.	Leaf Tisanes	Lemon balm, Mint, Lemon grass, French verbena
4.	Seeds	cardamom seeds
5.	Fruits	Cardamom, Caraway, Fennel.

Table 1: Medicinal plants parts used in tea

S.no.	Ingredients Use	Part	Family	Medicinal Name
1.	Green tea	Leaves	***Theaceae***	***Camellia sinensis***
2.	Giloy	Leaves	***Menispermaceae***	***Tinospora cordifolia***
3.	Moringa	Leaves	***Moringaceae***	***Moringa oleifera***
4.	Clove oil	Fruit	***Myrtaceae***	***Syzygium aromaticum***
5.	Cardamom	Fruit	***Zingiberaceae***	***Elettaria cardamom***
6.	Bay	Leaves	***laurels***	***Laurus nobilis***

Table 2: Composition of various polyherbal tea granules formulations

S.no.	Ingredients	F1	F2	F3	F4	F5
1.	Moringa –leaves	100 mg	105 mg	110 mg	115 mg	120 mg
2.	Tea –leaves	150 mg	150 mg	150 mg	150 mg	150 mg
3.	Bay –leaves	20 mg	15 mg	10 mg	20 mg	5 mg
4.	Cardamom –fruit	20 mg	20 mg	20 mg	-	5 mg
5.	Giloy –leaves	5 mg	5 mg	5 mg	15 mg	5 mg
6.	Clove –oil	5 ml	5 ml	5 ml	-	5 ml
7.	Calcium carbonate	--	--	--	--	10
8.	Starch (Rice broth)	q.s	q.s	q.s	q.s	q.s
9.	Bura (sugar)	q.s	q.s	q.s	q.s	q.s

2.2 Preparation of tea powder

Granules were prepared by using the wet granulation technique. Plant powder was taken according to composition in a mortar and mixed with fine granulated sugar. A sufficient quantity of rice broth was added to form a lumpy mass and then passed through the appropriate sieve to form granules. Granules were dried in the hot air oven.

2.3 Detection of phytoconstituents in tea powder[11,12]

2.3.1 Saponins detection

1 gm of the sample is taken with 20 ml of distilled water and shaken for 15 minutes in a graduated cylinder. A layer of foam, approximately 1 centimeter thick, then forms, indicating the presence of saponins.

2.3.2 Tannins detection

The sample (2 gm) is taken in a test tube with distilled water and stirred. Dilute ferric chloride 5% is then added dropwise, producing a blue or green-black color, indicating the presence of tannins.

2.3.3 Carbohydrates detection

The sample (1 gm) is taken with distilled water (3 ml) and then filtered. To the filtrate, 2-3 drops of Molisch's reagent are added slowly. Concentrated sulfuric acid is then added, forming a layer at the bottom. The formation of a violet ring at the junction of the two layers indicates the presence of carbohydrates.

2.3.4 Flavonoids detection

The sample (1 gm) is taken in a test tube, and a few drops of a dilute sodium hydroxide solution are added. A yellow color appears, indicating the presence of flavonoids.

2.3.5 Alkaloids detection

A few drops of aqueous hydrochloride are added to the sample before it is dried in a water bath and filtered. Thorough testing of the filtrate sample using Hager's reagent indicated the presence of alkaloids due to the formation of a yellow precipitate.

2.3.6 Proteins and amino acid detection

1 gm of the sample is dissolved in a few ml of water, and 2% ninhydrin solution is added. The test tube is then kept in a water bath for five minutes, resulting in a violet or deep blue color.

2.3.7 Terpenoid detection (Salkowski test)

5 gm of the sample is mixed with 2 ml of chloroform, and 3 ml of concentrated sulfuric acid is carefully added to produce a form layer. The presence of terpenoids is indicated by a reddish-brown color.

2.4 Preparation of granules

Granules were prepared by using the wet granulation technique. Plant powder was taken according to composition in a mortar and mixed with fine granulated sugar. A sufficient quantity of rice broth was added to form a lumpy mass and then passed through the appropriate sieve to form granules. Granules were dried in the hot air oven.

2.5 Evaluation of granules[13,14]

2.5.1 Bulk Density

A dry 100-ml measuring cylinder was volumeted with a 14gm sample mixture without being compacted. The sample was levelled carefully, and the apparent volume seemed steady (Vo). Determine the bulk density using this formula.

$$Pb = \frac{M}{V0}$$

Where bulk density = pb, weight of sample = M, and apparent volume of sample = V

2.5.2 Tapped density

After following the instructions for measuring the bulk density, the cylinder containing the sample was tapped 500 times at first, then 750 more times, until the difference between the succeeding measurements was less than 2%. The tapped volume (vf) was measured. Calculate the tapped density following the formula.

$$Ptap = \frac{M}{vf}$$

Whereas, tapped density = ptap, weight of sample = M, and tapped volume of sample = vf.

2.5.3 Carr's index

Carr's index is also known as the compressibility index. The following formula was used to calculate the percentage compressibility of the powder mixture based on the tapped density and the bulk density.

$$\text{Carr's index} = \frac{\rho tap - \rho b}{\rho tap} 100$$

Whereas bulk density is **ρ**b, tapped density is ptap. Lower Carr's index (<10) indicates excellent, 11–15 indicates good, 16–20 indicates fair, 21–25 indicates passable, 26–31 indicates poor, and 32–37 indicates very poor.

2.5.4 Hausner's Ratio

It serves as a proximate indicator for measuring powder flow. It is calculated by the following formula:

$$Hausner's\ ratio = \frac{tapped\ density}{bulk\ density}$$

where a lower Hausner's ratio (<1.25) indicates better flow properties than higher ones, between 1.25 and 1.5 showing moderate flow and more than 1.5 poor flow properties.

2.5.5 Friability

For the friability, the Roche friabilator method was used. Subjecting a quantity of granules to the modulated effects of abrasion and using a plastic chamber to shock that rotates at 25 rpm, dropping granules from a few inches away caused 100 revolutions to occur. Pre-weight granules were dusted and reweighted, and friability should be less than 1%, as per standard. The formula is used to determine it.

$$Fribility\ (\%) = \frac{Initial\ weight - final\ weight}{initial\ weight} 100$$

$$\frac{W1 - W2}{W1} 100$$

Initial weight is W1, and final weight is W2.

2.5.6 Moisture content[15]

A Petri plate is taken, and a 2 gm sample is placed in a preheated oven. The Petri plate is weighed and then dried at 130 °C for 2 hours in a hot air oven. After drying, the Petri plate is transferred to a desiccator to cool before being weighed again. The weight is determined, and the percentage of moisture content is calculated. This method is described by the AACC.

$$Moisture\ content\ (\%) = \frac{w1 - w2}{weight\ of\ sample} 100$$

weight of the sample before drying = w1.
The weight of the sample after drying equals w2.

2.5.7 Reconstitution index

5 g of each sample, taken from the ground sample of 50 cm^3, was dissolved in boiling water. The mixture was stirred for 90 seconds and transferred to graduated cylinders measuring 50 cm^3, and the amount of sediment was measured 30 minutes after settling[16].

2.5.8 Swelling index

Each sample was divided into three three-gramme portions (dry base) and placed in clean, dry, graduated cylinders measuring 50 cm^3. Volumes were recorded after the samples were gently leveled. For each sample, 30 cm^3 of distilled water were added. Every 15 minutes, the volume change (swelling) of the cylinder was measured while it was being rotated and left to stand for 60 minutes. The inflammation index was calculated as the initial volume-to-final volume ratio[16].

2.5.9 Disintegration time

The disintegration time of granules is determined by placing a specified number of granules into each test basket of a disintegration apparatus. The granules are immersed in a disintegration medium, typically distilled water or a specified buffer solution at 37°C. Timing is initiated simultaneously with the immersion of the test baskets. The time taken for all granules in each basket to completely disintegrate is observed and recorded. Disintegration is defined as the point where no residue of the granule remains on the mesh of the basket. Results are analyzed by calculating the average disintegration time based on recorded times for each batch of granules, and reporting follows method specifications or standard operating procedures (SOPs).

2.5.10 Determination of flavonoids

1 gm of plant extract is taken in a test tube and 4 ml of methanol is added to dilute it (standard), followed by spinning. Then, 1 ml of $AlCl_3$ is added to the sample and quickly spun. Next, 1 ml of 1 molar sodium acetate is added, and the sample is incubated at room temperature (RT) in the dark for 45 minutes. After incubation, absorbance is measured at 420 nm. Quercetin is used as the positive control at comparable concentrations. The amount of flavonoids in the extract is calculated as mg quercetin equivalent per gram (QE)[17].

2.5.11 Determination of tannins

The standard tannic acid solution (µg) is mixed with 1 gm of the sample, and the volume is adjusted to 7.5 ml using distilled water. Then, 0.5 ml of Folin-Denis reagent and 1 ml of 7.5% Na2CO3 are added, and the volume is made up to 10 ml with distilled water. Absorbance is measured at 700 nm. The amount of tannin is estimated as mg of tannic acid equivalent (TAE) per gram of extract[18].

2.5.12 Determination of Total Free Amino Acids

1 ml of ninhydrin solution is added to 0.1 gm of sample. The volume is brought up to 2 ml by adding distilled water. The tube is heated in a boiling water bath for twenty minutes. To the contents, 5 ml is added to dilute, then shaken. After 15 minutes, the intensity of the violet color is checked with a colorimeter at 570 nm using a reagent blank. The color remains constant for an hour. When preparing the reagent blank as above, 0.1 ml of 80% ethanol is taken in place of the extract[19].

2.5.13 Determination of total carbohydrate

In a boiling tube, 100 mg of the sample is measured out. It is placed in a water bath with 5 mL of 2.5 N HCl and hydrolyzed for 3 hours. After hydrolysis, the sample is cooled to room temperature. Effervescence stops after neutralizing with solid sodium carbonate. The volume is adjusted to 100 ml before centrifugation.

For preparation of the test samples and standards:

- Working standard is pipetted into test tubes in amounts of 0.2, 0.4, 0.6, 0.8, and 1 ml.
- Two separate test tubes are prepared with 0.1 ml and 0.2 ml of the sample solution.
- Water is added to bring the volume in each tube to 1 ml. A blank is created with 1 ml of water.

To each tube:

- 1 ml of phenol solution is added.
- 5 ml of 96% sulfuric acid is added and shaken well.

After shaking, the tubes are submerged in a water bath set at 25 to 30 °C for 20 minutes. The color intensity is measured at 490 nm using a spectrophotometer[19].

2.5.14 Determination of terpenoids

100 mg of dried plant are taken with 9 ml of ethanol. The mixture is soaked for 24 hours and filtered. To obtain the filtration, 10 ml of petroleum ether is added using a separating funnel. The ether extract is divided into pre-weighed glass vials and allowed to fully dry. The yield of the total terpenoid contents is calculated using the formula after the ether has been evaporated[20].

$$\textbf{\textit{Total terpenoid contents}}\ (\%) = \frac{\boldsymbol{wi - wf}}{\boldsymbol{wi}}\mathbf{100}$$

2.5.15 Determination of saponin

1 mg of sample was taken and dissolved in 80% methanol, and 2 ml of vanillin was subsequently added to the ethanol and thoroughly mixed. A solution of 2 ml of 72% sulfuric acid was then added and subjected to heat treatment in a water bath at 60 °C for a period of 10 minutes. The absorbance was subsequently measured at a wavelength of 544 nm relative to the reagent blank[21].

2.5.16 Determination of alkaloids

1 mg of the plant extract was dissolved in Dimethylsulfoxide. Then, 1 ml of 2N HCl was added before filtering. A solution was prepared by adding 5 ml of bromocresol green solution and 5 ml of phosphate buffer to the solution in a separate funnel. The mixture was vigorously shaken with 1, 2, 3, and 4 ml of chloroform before being collected in a 10 ml volumetric flask and diluted with chloroform to the volume. Reference standard solutions of atropine were made by repeating the same process as previously described, resulting in solutions of 20, 40, 60, 80, and 100 g/ml. The absorbance of standard solutions and test solutions was measured at 470 nm on the reagent blank using an UV/visible spectrophotometer. The absorbance was used to measure alkaloids in milligrams of active ingredient per gram of sample[22].

2.5. 17 Stability studies

Only through stability studies can the efficacy of a formulation be assessed. The goal of stability is to produce a product that, under specified storage conditions and peak profiles, will remain safe and effective through the end of its shelf life. Accelerated stability tests on the drug's improved formulation were conducted for three months at the predetermined temperatures and relative humidity levels of 25°C/60% RH and 30°C/60%. After three months, the samples were visually examined for any colour changes brought on by physical and chemical interactions between the excipients and the drug[23].

3. Results and Discussion

Lifestyle has vital importance in the current scenario. Today, due to pollutants and imbalances in working lifestyles, lifestyle disorders need attention in the healthcare system. Lifestyle disorders like obesity, hair fall, stress, CNS, skin, and ageing-associated disorders. Thus, a polyherbal formulation is of vital importance. In this study, we design and formulate polyherbal granules to satisfy the needs of people. Polyherbal formulations were composed of different herbs. Natural herbs are known for their vast pharmacological properties due to their multiple active constituents. The design of herbal granule formulations has the objective of targeting lifestyle disorders. The polyherbal granule formulation has the advantage of being used as a tea drink. The herbs used in this formulation are moringa, tea leaves, giloy, bay leaves, cardamom, and clove oil. Moringa main has quercetin, gallic acid, betasitosterol, campeferol, and luteolin. Tea leaves mainly have caffeine, flavine, and gallic acid. Giloy's main content is berberine, choline, calcium, and phosphorus. Bay leaves have monoterpenes and flavones. Cardamom has carotenoids, coumarins, avenasterols, and sitosterols; clove has eugenol. Gallic acid, caffeine, theaflavins, berberine, flavone, and coumarin are water-soluble polyphenols and have significant anti-oxidant activity, as shown in Table 9. The formulation has water-soluble antioxidants that reduce oxidative stress in cells and, as a consequence, have anti-ageing, anti-cancer, skin-nourishing, and immunomodulatory effects. The formulation is to be evaluated by determining phytoconstituents, as shown in Tables 4,5 and 6. The granules are to be designed, formulated, and evaluated with different parameters based on their composition, as shown in Table 6. The granules were evaluated by moisture content, swelling index, reconstitution, stability study, and disintegration test, as shown in Table 8. The granules undergo sensory evaluation by colour, odour, and taste, as shown in Table 7.

The formulation is orally administered with hot water, and its anti-oxidant response is due to water-soluble constituents such as gallic acid and flavone. Mood elevator, diuretic, and stimulant effects due to the flavine. It aids in hypertension as a diuretic due to berberine. Muscle and bone strength are enhanced by calcium and phosphorus, which occur in giloy.

Table 4: Phytochemical class of constituent of tea

Phytochemical	Moringa	Tea	Giloy	Cardamom	Tejpatta	Clove oil
Saponin	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Amino acid	+	-	+	-	+	+
Flavonoids	+	+	+	+	+	+

(Detected = +, Not detected = -)

Table 5: Phytochemical class of constituent of formulated herbal tea granules

Phytochemicals	F1	F2	F3	F4	F5
Saponin	++	+	+	++	+
Tannin	+	++	+	+	+
Carbohydrates	+	+	+	+	+
Amino acid	+	++	+	+	+
Flavonoids	+	+	+	+	+
Terpenoids	+	+	+	+	+

(Detected = +, More detected = ++, Not detected = -)

Table 6: Evaluation of formulated herbal tea granules

Evaluation	F1	F2	F3	F4	F5
Bulk Density	0.411±0.1	0.4±0.1	0.421±0.1	0.4±0.2	0.411±0.1
Tapped Density	0.528±0.1	0.538±0.1	0.535±0.1	0.569±0.1	0.518±0.1
Carr's index	22.348±0.2	25.650±	15.814±0.1	28.571±0.2	20.656±0.1
Hausner's Ratio	1.284±0.2	1.345±0.2	2.375±0.1	1.424±0.3	1.260±0.1

Values are the mean± standard deviation of triplicate determinations. Values with the same consecutive exponent are not significantly different at the P > 0.05 level.

Table 7: Sensory evaluation of formulated tea granules

Formulations	F1	F2	F3	F4	F5
Colour	Dark green	Dark green	Dark green	Dark green	Dark green
Colour of brew	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Light Yellowish-brown
Taste	Mild	Mild	Mild	Mild	Mild

Table 8: Evaluation parameters of the formulated tea granules

Evaluation	F1	F2	F3	F4	F5
Disintegration	9 min	9.5 min	10 min	11 min	8.5 min
Moisture content	12.7±0.09	9.70±0.05	13.5±0.07	14.5±0.02	11.5±0.08
Stability study	Stable	Stable	Less Microbial growth	Microbial growth	Less microbial growth
Reconstitution index	10.06±1.0	10.04±1.0	10.02±0.8	9.99±1.0	10.3±1.0
Swelling index	0.35±0.2	0.45±0.1	1.15±0.2	0.78±0.1	0.65±0.1

Values are the mean± standard deviation of triplicate determinations. Values with the same consecutive exponent are not significantly different at the P > 0.05 level.

Table 9: Quantitative value of phytoconstituents in the formulated tea granules

Phytoconstituent	F1	F2	F3	F4	F5
Saponin	8.251±0.02	8.951±0.09	7.589±0.05	7.652±0.04	7.165±0.04
Tannin	0.075±0.001	0.055±0.01	0.050±0.01	0.069±0.01	0.049±0.02
Carbohydrates	0.251±0.01	0.341±0.02	0.386±0.02	0.269±0.01	0.410±0.01
Amino acid	0.412±0.02	0.421±0.01	0.429±0.01	0.451±0.01	0.442±0.01
Flavonoids	25.25±0.09	32.80±0.15	26.97±0.12	27.56±0.08	20.32±0.04
Terpenoids	60.54±2.00	62.52±1.01	66.65±1.90	69.85±1.04	68.52±2.12

4. CONCLUSION

The current polyherbal granule formulations are composed of bioflavonoids and other pharmacologically active natural herbs. Evaluation of formulations reveals their better dispensability, stability, life span, and acceptability in hot water.

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