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Research Study

Formulation of Herbal Green Tea Using *Terminalia Arjuna* Bark, *Glycyrrhiza Glabra* Root, *Gymnema Sylvester* Leaf And *Green Cardamom* Seeds: Its Nutritional & Phytochemical Analysis

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ABSTRACT

Tea in general and green tea in particular are gaining market attention due to a growing awareness of health benefits derived from their use, but work in the production of flavored green tea products is limited. The objectives of the study were to conduct Nutritional analysis, phytochemical analysis, antioxidant and antibacterial activity of *Terminalia arjuna* bark, *Glycyrrhiza glabra* root, *Gymnema sylvester* leaf, Green Cardamom seeds, Green tea and formula mixture of these herbs to evaluate their potential for the development of new herbal tea. These herbs are a rich source of natural antioxidants and polyphenols. Total phenolics and flavonoids contents were obtained for methanolic extracts for each herb sample. Antioxidant activities of the extracts were estimated using DPPH radical scavenging activity and FRAP assay. Formulation mixture is an excellent sources of calcium, magnesium and phosphorus. The methanolic extracts showed good antibacterial activity in *Bacillus subtilis, Bacillus cereus* and *E. coli.* The formulation of green tea with these herbs has been completed, which gives green tea colour, fragrance, flavor, astringency and overall acceptability and gives many health benefits.

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INTRODUCTION:

The Tea is the most consumed beverage in the world, but its origination is attributed to china [1] and becomes as an important food product in the world. After china, the 2nd largest producer of tea is India [2]. Consumers of these days are concerned about their nutrition and they demand more organic and health-friendly food so tea seems to be a good vehicle in this respect due to its good taste and aroma. Tea is therefore part of a rapidly growing demand for wellness drinks [3].

Traditionally, Tea is historically graded as green, Oolong, black and herbal tea [4] and can be categorized at the time of manufacture based on their processing stages. *Camellia sinensis* is the plant from which green tea and all kinds of tea are made. Fermentation is not done during green tea Processing. Green tea imparts several health promoting components because of its high range of phenolic compounds. Green tea is covered to deliver nearly 4000 bioactive compounds under which one third is polyphenols [5].Tannins and flavonoids are the important polyphenols present in the Green tea. Catechins, one of the important flavonoids present in the green tea which is also known as vitamin P [6]. Green tea imparts several health benefits like weight loss, diabetes, heart diseases, Alzheimer's and Parkinson's, blood pressure, depression, anti-viral, skin care.

There are many flavored green teas. Popular flavored green teas are lemon green tea, ginger & mint green tea, lemon honey green tea, jasmine green tea, etc. On the basis of some unpublished reports, however, green tea imparts poor in sensory appeal due to the lack of distinct Flavor properties. Therefore, it may be needed to blend green tea with other herbs as a means of improving its sensory appeal and for good health and wellness. The herbs discussed in the research work are *Terminalia arjuna* bark, *Glycyrrhiza glabra* root, *Gymnema sylvester* leaf and Green Cardamom seeds.

Terminalia arjuna is a tree in which its bark is having good medicinal properties. It acts as a cardio tonic. It is helpful to lower down the blood pressure and pulse rate, and may boost up the aerobic exercise capacity. Major chemical present in the bark is saponins, flavonoids, tannins and phytosterols. It has antibacterial antimutagenic, antioxidant, hypolipidemic, and hypocholesterolaemic and anti-inflammatory effects [7].

Glycyrrhiza glabra Linn. belonging to family Fabaceae, known as mulethi or licorice. It is used in many systems of medicines including Unani, Ayurveda. Homeopathy, Chinese and Siddha to cure various types of complications like hepatitis, ulcers, pulmonary, skin diseases etc. Traditionally mulethi is used as mild laxative, anti-arthritic, anti-inflammatory, anti-biotic, anti-viral, anti-ulcer, memory stimulant (being MAO inhibitor), anti-tussive, aphrodisiac, anti-mycotic, estrogenic, anti-oxidant, anti-caries agent, anti-neoplastic, anti-cholinergic, anti-diuretic, hypolipidemic agent. It constituted phytoconstituents such as glycyrrhizin, glycyrrhizinic acid, glabrin A&B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols [8].

Gymnema sylvestre (Asclepiadaceae) also known as 'gurmar' or 'sugar destroyer' is a woody, climbing traditional medicinal herb that has many therapeutic uses in Ayurvedic system of medicine. It is used for lowering serum cholesterol, triglycerides and blood glucose level (hypoglycemic or antihyperglycemic), hypolipidaemic, weight loss, stomach ailments, constipation, water retention and liver diseases. The flower, leaves and fruits include alkaloids, flavones, saponins, sapogenins, anthraquinones, hentri-acontane, pentatriacontane, α and β-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin-related glycosides and stigmasterol. Gymnemic acids are believed to be responsible for their antidiabetic function and to induce insulin release from the pancreas is the major component of an extract. [9].

The aim of the present study is to evaluate the nutritional, phytochemical constituents, antioxidant and antibacterial activity of *Terminalia arjuna* bark, *Glycyrrhiza glabra* root, *Gymnema sylvester* leaf, Green Cardamom seeds and Formulation mixture and to blend these herbs with the Green tea.

MATERIALS AND METHODS

Sample collection

Terminalia arjuna bark, *Glycyrrhiza glabra* root, *Gymnema sylvester* leaf, Green Cardamom seeds,

Green tea were collected from a local market of Bareilly, Uttar Pradesh. All herbs were carefully inspected and all foreign materials removed and thoroughly washed under tap water. The clean sample was dried in an oven at 60°C and coarsely powdered using a mixer grinder, sieved and then stored in air-tight, light resistant container for further use. The samples were labelled as the *Terminalia arjuna* bark (TA), *Glycyrrhiza glabra* root (GG), *Gymnema sylvester* leaf (GS), Green Cardamom seeds (GC), Green tea (GT) and Formulation mixture (F MIX) respectively.

Sample extraction

About 25g each of the powdered herb samples (TA, GG, GS, GC) and 50g of powdered F MIX of these herbs was successively extracted with 100 ml and 200 ml of methanol respectively. The mixture was incubated at 60°C at 150 rpm for 24 hrs in an incubator shaker. The mixture was filtered through Whatman paper to obtain the filtrate. The residue left after extraction was mixed with another 100 ml and 200 ml methanol respectively, and then incubated again at 60 °C at 150 rpm for 24 hrs in an incubator shaker for 24 hrs and then both the combined filtrates obtained were then transferred to respective labelled beakers and covered with foil, with fine pores for the solvent to evaporate at 60°C in a hot air oven to afford methanol extracts.

Nutritional Analysis

Determination of Moisture content

The moisture content was measured described by AACC [10] method. Two gram sample was placed in a preheated and weighed glass petriplate and then dried in a hot air oven at 130 °C for 2 hrs or till constant weight after drying glass petriplate was transferred to the dessicator to cool and then petriplate was reweighed. The weight loss was calculated as a percentage of the amount of moisture present in the sample drug.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{\text{Weight of Sample}} \times 100$$

W1 = Weight (g) of Sample before drying.
W2 = Weight (g) of Sample after drying.

Determination of Ash content

The ash content was measured, described by AACC [10] method. 2 gram of sample was placed in a preweighed crucible and then it was allowed to incinerate in a muffle furnace at 820 °C for 4 hours and then crucible was cooled in a dessicator and then weighed.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of Sample}} \times 100$$

Determination of mineral composition

The experimental method used to detect trace metals is inductively coupled plasma optical emission spectrometry (ICP-OES). It is a method of emission spectroscopy which uses the plasma coupled inductively to generate excited atoms and ions which emit electromagnetic radiation at a particular element's characteristic wavelengths.

It is a flaming technique with a flaming temperature of between 6000 and 10000 K. It is also a solution technique & standard silicate dissolution methods are employed [11]. This emission frequency is

representative of the component concentration within the sample.

Phytochemical analysis

Determination of Total Phenolics

Total phenolics were determined using the Folin–Ciocalteu assay [12]. The absorbance was measured at 765 nm and the results were expressed in gallic acid equivalents.

Determination of Total Flavonoids

Total flavonoids were estimated using Aluminiun Chloride colorimetric method [13]. The Absorbance was measured at 510 nm. Results were expressed in Catechin equivalents.

Determination of Crude alkaloids

Crude alkaloid was determined gravimetrically for phytochemical analysis [14] with some modification. The alkaloid content was calculated as a percentage.

$$\% \text{ of Alkaloid} = \frac{\text{Final Weight} - \text{Initial weight}}{\text{Sample Weight}} \times 100$$

Determination of Tannins

Tannins were estimated according to the method described by Van-Buren and Robinson [15]. Absorbance was measured at 605nm and the result was expressed in Tannic Acid equivalents.

Qualitative phytochemical analysis

The methanolic extracts of TA, GG, GS, GC, GT & F MIX were subjected to different chemical tests for the detection of phytoconstituents such as terpenoids, phytosterol, coumarin, anthraquinones, and phlobatannins.

Test for identification of Terpenoids

5 mg of the methanol extract was mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid. The layer interface was observed for reddish brown coloration which indicates the presence of terpenoids [16].

Test for identification of Coumarins

10 mg of the extract is dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolorizes while adding conc. HCl shows the presence of coumarin [17].

Test for identification of phytosterols

Small quantities of the extracts were dissolved in 5 ml of chloroform separately then chloroform solution was subjected to the Salkowski test in which 1 ml of above prepared chloroform solution and a few drops of conc. H_2SO_4 were added. Brown colour revealed the presence of phytosterol [16].

Test for identification of phlobatannins

80 mg of plant extract was boiled in 1% HCl, the deposition of a red precipitate indicated the presence of phlobatannins [18].

Test for identification of anthraquinone

Two hundred mg of fraction was boiled with 6 ml of 1% HCl and then filtered. Then the filtrate was shaken with 5 ml of benzene. Layer of benzene was removed and 10 % ammonium hydroxide was added. Pink, violet and red in alkaline phase indicates the presence of anthraquinones [19].

Anti oxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was analyzed by the method of Blois [20] with slight modification. Two mg (200 PPM) of sample extract was dissolved in 10 mL methanol and then from this 10 mL, 1mL of sample was taken and dissolved with 1mL of a 0.3 Mm methanol solution of DPPH (2, 2-diphenyl-1–picrylhydrazyl) plus 1 mL methanol in a test tube. After this test tube was kept in the dark for 10 minutes. Blank was prepared without the extract. Methanol was used as a reference. Then absorbance was taken at 517nm.

A radical scavenging activity was expressed by % of scavenging activity and was calculated by the following formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{OD Blank} - \text{OD Sample}}{\text{OD Blank}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined by Benzie and Strain [21] method. The FRAP reagent was prepared fresh each day by mixing 2.5 mL of TPTZ (10 mM in 40 mM hydrochloric acid), 2.5 mL of ferric chloride (20 mM) and 25 mL of sodium acetate buffer (300 mM, pH 3.6). A 100 µl of extract was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 °C for 4 min. The absorbance was measured at 593 nm and the result was expressed as BHT equivalent.

Determination of antibacterial activity

The antibacterial activity of the Methanolic extract of F mix was evaluated by agar well diffusion method against three Gram positive bacteria and two Gram negative bacterial test pathogens [22]. Extracts were reconstituted to a final concentration of 100mg/ml and 500 mg/ml. DMSO and Methanol were used as control in one of the wells. Nutrient agar was inoculated for each bacterial strain by spreading each strain 100 µl in the respective plate. Wells of 6 mm diameter were punched on the agar plates and 100 µl of extracts were loaded into the wells. The plates were incubated at 37 °C for 24 hrs. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported on the scale of millimeters (mm).

Preparation of Formulations

The five dried and milled herbs (TA, GG, GS, GC & GT) were mixed in varying proportions to obtain three different formulations Table 1, 1.75 gram samples of each formulation were bagged in rectangular infusion tea bags. Commercial Green tea (Tetley Company) was used as a control. They were labelled accordingly for sensory analyses.

Table 1: Proportion of herbs in blended product

Herbs	Formulation 1 (%)	Formulation 2 (%)	Formulation 3 (%)	Control
GT	30	40	50	100
TA	25	15	15	-
GG	20	20	15	-
GS	20	15	15	-
GC	5	10	5	-

RESULTS AND DISCUSSION

Nutritional analysis

During nutritional analysis, moisture content and ash analysis are very important as they directly affect the food's nutritional content, its stability and processing, etc. The moisture and ash contents were calculated for F MIX which are reported in Table 2. The mineral components were analyzed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). The quantity of minerals present in the F MIX is as tabulated in the Table 3. The good amount of calcium was present in F MIX which can play a vital function in rigidity to the bones as well in the neuromuscular functions, blood clotting, treatment of osteoporosis [23] whereas magnesium has multiple health benefits including transmission of nerve impulses, detoxification, body temperature regulation, energy production, heart diseases [24] and the development of fit bones and teeth. Another one is phosphorous, which were present in high amount. Phosphorus potency involves good bone growth, enhanced digestion, controlled excretion, improved

energy absorption, hormonal balance, protein production, cellular repair, as well as essential acts for various parts of the body such as the brain, liver, heart and blood [25].

Table 2: Ash and Moisture content estimation for F MIX

Constituents	(F MIX)
Moisture	5%
Ash	8.1%

Table 3: Mineral content estimation for F MIX

S.No.	Analyte	Concentration (ppm)
1	Ca	9985.50
2	Fe	173.75
3	Mn	167.50
4	Mg	1040
5	Ni	2.13
6	P	13925.0
7	Sr	45.0
8	Zn	15

Phytochemical analysis

Phenolics and Flavonoids are a wide class of chemical compounds found in plants. They impart quality and nutritional value and plays a vital role in human fitness such as anti -inflammatory [26], antidiabetic [27], antiviral [28], antioxidant [29], Therefore, total phenolic and flavonoids content of different methanolic extracts of herbs were estimated Table 4. Crude alkaloids and tannin are the compounds present in plants. Tannins are polyphenols that are responsible for the astringent flavor of food and shows anti-carcinogenic [30] and antimicrobial properties where as small doses of alkaloids has a therapeutic effect as muscle relaxants, pain killers and antimicrobials in human beings [31]. Therefore, crude alkaloid and tannin content of different samples of herbs were estimated Table 4.

Table 4: Polyphenolic compounds of different methanolic extracts

Herbal extracts	GAE equivalents (µg GAE/ mg sample)	Catechin equivalents (µg CE/ mg sample)	Alkaloids (%)	Tannic acid equivalents (µg TAE/ mg sample)
TA	41.25	84	2.016	0.0785
GG	7.8	24	0.804	0.1235
GS	4.68	42	0.368	0.2985
GC	3.43	120	1.172	0.2985
GT	30.31	58	0.672	0.2335
F MIX	18.43	50	1.56	0.271

Qualitative phytochemical analysis

Methanolic extracts of TA, GG, GS, GC, GT & F MIX were also subjected for qualitative tests such as phytosterol, terpenoids, anthraquinones, coumarin and phlobatannins in which all the herbs show the presence of phytosterol and terpenoids in Table 5. Phytosterols are very beneficial in lowering cholesterol levels and reduces coronary heart diseases [32] while terpenoids have a unique antioxidant activity [33]. Coumarins were found to be

present only in GG and F MIX which shows the anti-fungal and anti- tumor activity.

Table 5: Qualitative analysis of herbal extracts to screen the presence of phytochemicals

Sample	Phytosterol	Terpenoids	Phlobatannins	Anthraquinone	Coumarin
TA	+	+	_	_	_
GG	+	+	_	_	+
GS	+	+	_	_	_
GC	+	+	_	_	_
GT	+	+	_	_	_
F MIX	+	+	_	_	+

Antioxidant activity Analysis

DPPH radical scavenging activity

This process is most frequently used in many herbal plants to display antioxidant activity .DPPH is a stable, violet-colored free radical. If free radicals have been scavenged, DPPH will change its colour from violet to pale yellow or colorless. This property allows visual monitoring at 517 nm. A scavenging activity in % inhibition of methanolic herbal extracts is given in Table 6. Antioxidants are working as a protection of cells against the destructive effects of reactive oxygen species (super oxide or hydroxyl radicals). The reaction between antioxidants and reactive oxygen species results in oxidative stress causes cellular damage [34]. Oxidative stresses have been related to cancer, aging, atherosclerosis, inflammation, ischemic injury and Neuro degenerative diseases [35].

Table 6: DPPH assay of different herbal extracts

Analyte	% Inhibition
TA	87.93
GG	45.46
GS	51.95
GC	38.91
GT	94.10
F MIX	93.23

Ferric reducing antioxidant power (FRAP) assay

This approach is based in the presence of antioxidants on the concept of reducing the complex of ferric tripyridyl-s-triazine to ferrous colored shape. The antioxidants present in the samples reduce the complex of ferric tripyridyl-s-triazine to form a complex of blue colors resulting in an increase of 593 nm in absorbance. The antioxidant activity of different methanolic herbal extracts is given in Table 7.

Table 7: FRAP assay of different herbal extracts

Analyte	BHTE equivalents(µg BHTE/mg extract)
TA	2930
GG	961
GS	861
GC	1876
GT	2223
F MIX	2930

Antibacterial activity

Four gram positive *(Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and two gram negative (*E. coli*, *Salmonella enterica*) bacteria were used to determine the antibacterial activity of methanolic extract of F MIX. Agar well diffusion method was used to determine the activity against the bacteria by measuring zone of inhibition. Inhibiting concentrations used for the extract was 100mg/ml and 500 mg/ml. Antibacterial activity was analyzed against the DMSO and methanol solvents. Table 8 lists the clearance area or region of methanolic extract inhibition of the F MIX against specific bacterial growths.

Table 8: Antibacterial activity of F MIX against various bacteria using the agar well diffusion method

Test pathogens	Diameter of zone of inhibition (mm)			
Bacillus subtilis	25	14	28	15
Bacillus cereus	22	15	23	19
Staphylococcus aureus	15	11	21	15
Staphylococcus epidermidis	15	1	20	11
E. coli	23	14	22	13
Salmonella enterica	22	15	23	19

CONCLUSION

The results of the nutritional, phytochemical, antioxidant and antibacterial activity showed that *Terminalia arjuna* bark*, Glycyrrhiza glabra* root, *Gymnema sylvester* leaf and *Green Cardamom* seeds, Green tea and the formulation mixture of these herbs showed that they can be proven to be an excellent source of nutraceuticals and Flavoring agents. Multiple health benefits featured in the blended formulation make it a perfect physical and psychological health Rejuvenator. Although several health benefits are also credited to green tea, but according to some unpublished reports it has been observed that the sensory appeal of green tea is not much attractive due to the lack of distinct flavor properties. It may therefore can be a good idea to combine green tea with other herbs (*Terminalia arjuna* bark*, Glycyrrhiza glabra* root*, Gymnema sylvester* leaf and *Green Cardamom* seeds) for developing flavored green tea, which not only adds to its appeal, but also palatability & thereby making it a wonder product in the context of human health. As sensory appeal matters the most to consumers more than health or nutritional benefits, so the above infusion will provide them with new alternatives to traditional flavored teas which can impart health benefits too.

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