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Formulation of Effervescent Tablet from Lyophilized Extract of *Camellia sinensis* Using Stevia as a Natural Sweetener

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Abstract

The objective of the present study is formulation and optimization of effervescent tablets of green tea extract. Health benefits of green tea such as antioxidant, anti-diabetic, anti-cancer, antihypertensive, antimicrobial, anti-inflammatory, coronary heart disease, etc., are attributed to the presence of catechins, particularly (-)-epigallocatechin-3-gallate, as indicated by *in-vitro*, *invivo* as well as clinical studies. Extraction of green tea extract, standardization of extract, formulation of tablets and optimization of formulation were carried out. Physicochemical and phytochemical screening were performed. Estimation of polyphenolic content and flavonoid content in lyophilized extract of green tea was carried out. The formulation F5 passed all the evaluation test parameters with shortest effervescent time, better hardness, and friability results. One tablet of our formulation can act as a substitute for one cup of green tea.

Keywords: Effervescent, Catechins, Polyphenols, epigallocatechin-3-gallate, Green Tea, Tablet.

Introduction

Herbal 'renaissance'is happening all over the globe. About 30% of the plant species are used for medicinal purposes.(1) Green tea is amongst them and is highly beneficial in the elimination of free radicals. It is the second most consumed beverage in the world. India ranks second in terms of tea production after China. The global market for green tea supplements is anticipated to be worth US$ 5.7 billion in 2023 and is predicted to grow at a compound annual growth rate (CAGR) of 6.1% from 2023 to 2033. During the COVID-19 pandemic, demand for green tea supplements increased as more individuals looked for foods high in components that improve immunity. The National Institutes of Health (NIH) reports that scientists are conducting research to determine whether taking green tea supplements could increase mental acuity and stave off a deterioration in cognitive function. Like coffee, green tea has a high caffeine content that gives drinkers a brief energy boost.(36)

Catechins are the active compounds responsible for beneficial activity of green tea.(2) Catechins outperform Vitamin C and beta-carotene ten times in scavenging the alkyl peroxyl radical.(3) Study on green tea reveal polyphenols to be more potent antioxidants than Vitamin C, Vitamin E, rosemary extract, and even curcumin in some systems.(4) The health benefits of Green tea are: antioxidant, antimicrobial, antihypertensive, anti-inflammatory, in cardiovascular health, anti-cancerous and anti-diabetic.(5) Effervescent tablets provide rapid onset of action and are more stable than other dosage forms. Encapsulation of green tea extract in the form of effervescent tablets provides several advantages and health benefits to patients.(6)

Pharmacological Importance of Green Tea

Green tea,which is extracted from various parts of*Camellia sinensis*is a rich source of bioactive compounds, particularly polyphenols, and has been associated with various health benefits. (7)Phytoconstituents found in green tea extract are catechins (epigallocatechin gallate EGCG). These constituents act as potent antioxidants and anti-inflammatory agents. (8)Catechins have been shown to modulate inflammatory pathways, potentially reducing inflammation associated with chronic diseases.It may be useful in treatment of high blood pressure and reduce cholesterol levels. It may also help in lowering risk of heart diseases and incidence of heart attack by improving vascular function. The antioxidant and anti-inflammatory pathway involved in mechanism of green tea extract may also exhibit neuroprotective effect hence proving its importance in mitigation of neurological disorderslike depression, cerebral ischemia, multiple sclerosis, Parkinson, and Alzheimer'sdisease. (9)

Table 1: Summarization of Preclinical studies on *Camellia sinensis*

S.L. No	Species	Dose	Duration	Result	Ref.
1.	Mouse	0.1%	84 days	Decrease in tumor progression in duodenum.	Yamane, T., et al, 1996.
2.	Rat	0.01%	112 days	Decrease in the number of tumors in colon.	Mazhar et al., 2021
3.	Rat	2% w/v	224 days	Decrease in number of tumors, Ras-p21 and Bcl-2 expression and tumor volume.	Jia, X. D., et al, 2000.
4.	Hamster	$6.0gL^{-1}$	126 days	Decrease in the number and volume of oral tumors.	Li, N., et al, 2002.
5.	Rat	2.5%	35 days	Decrease in LDL cholesterol, LDL	Yokozawa, T.,

				peroxidation and increase in HDL.	et al, 2002.
6.	Mouse	0.5% w/w	308 days	Decrease in insulin, plasma glucose, and leptin.	Murase, T., et al, 2002.
7.	Rat	$3gL^{-1}$	35 days	Increase in GSH and total antioxidant.	Skrzydlewska, E., et al, 2002.
8.	Rat	20mg/kg	40 days	Decrease in creatinine level in the serum and urea in the blood.	Yokozawa, T., et al, 1999.
9.	Rat	5.0%	91 days	Decrease in body weight.	Satoh, K., et al, 2002.
10.	Rat	2% v/v	42 days	Increase in CYP1A1 and CYP1A2 activity.	Sohn, O. S., et al, 1994.

Mechanism of Action

The mechanisms of action of green tea extract are due to abundance of bioactive components extracted fromit, including polyphenols, catechins, and flavonoids.[10]Epigallocatechin gallate (EGCG) is the main active component in green tea extract and is responsible for many of its health benefits.Polyphenols in green tea, particularly EGCG, act as potent antioxidants. They help neutralize free radicals in the body, reducing oxidative stress and preventing cellular damage.[11]The chemistry underlying this activity is mainly the consequence of single electron transfer, hydrogen atom transfer reactions (HAT), or both involving hydroxyl groups.[12]Green tea extract's mode of action may be linked to more extensive ROS removal via enhancing the brain's activity of antioxidant enzymes like catalase, glutathione reductase (GSH-Rd), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD). Green tea extract also contains antineoplastic properties. The mechanism involved in such activities include inhibition of gene transcription of the responsible factor and activation of tumor suppressing genes.[13]

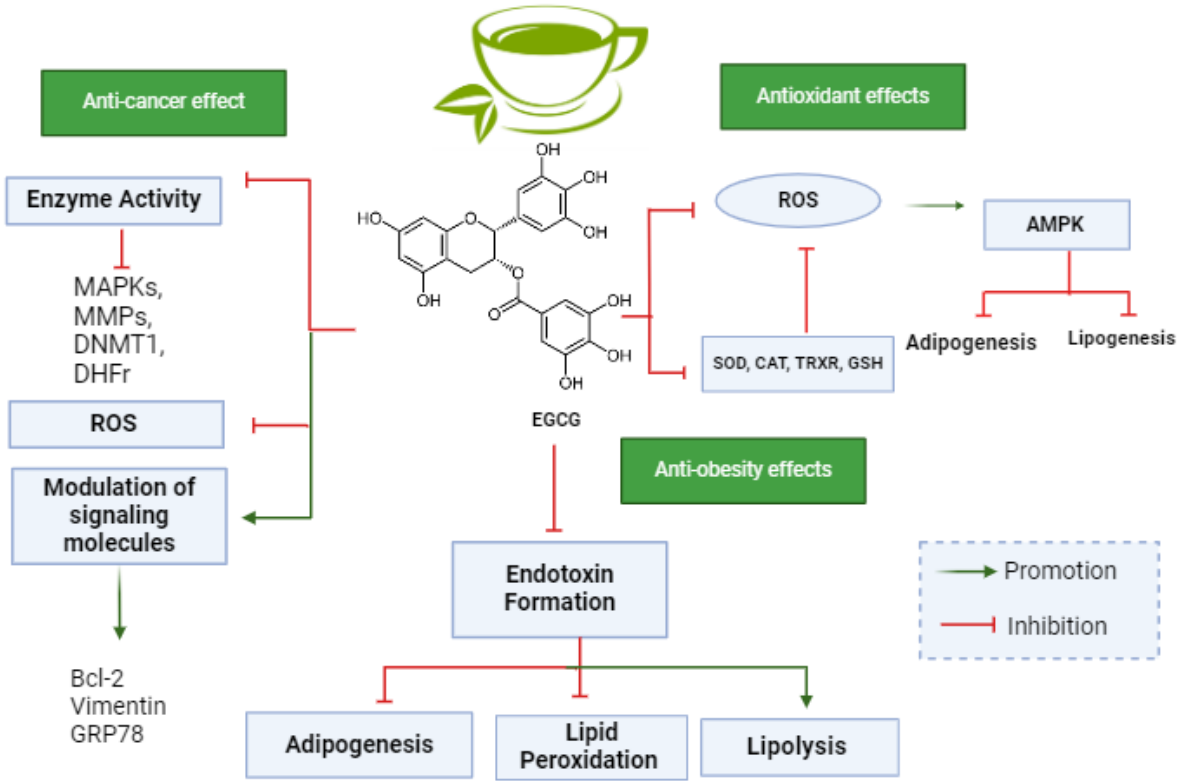


Figure 1: Various pathways promoted or inhibited for pharmacological activity of *Camelliasinensis*

Myths:Green tea (*Camelliasinensis*) and Weight Management Correlation

Green tea contains various polyphenols and phytoconstituents such as catechins (EGCG) and caffeine that increases metabolic rate and aids in weight loss. The catechins in green tea may enhance fat metabolism in humans, especially during physical activity.[(14)] This could contribute to improved fat oxidation and, in turn, support weight loss efforts. It is not a miracle solution, but may offer some benefits when combined with a healthy diet and regular exercise. [(15)]The myth likely arises from the fact that green tea contains compounds called catechins, particularly epigallocatechin gallate (EGCG), which some studies suggest may have a modest impact on metabolism and fat oxidation.[(36)]. However, the effects are not substantial enough to replace other essential aspects of weight management, such as maintaining a calorie-controlled diet and engaging in regular physical activity.[(16)]Phytoconstituent of green tea were found to have hepatotoxic and renal damaging effects in experimental animals.The exact mechanism by which green tea causes liver damage is unknown, however it may be due to EGCGor its metabolite EGCG, which can cause oxidative stress and liver damage under specific circumstances.[(17)]Hepatotoxicity caused by green tea expresses in form of jaundice and acute viral hepatitis which improves rapidly when the consumption of the supplement is stopped.The United States Pharmacopoeia (USP) has conducted safety studies and found adverse effects after the use of high-dose green tea extract preparations, most of which were liver injury reports.[(18)]For example, male CF-1 mice treated with high-dose EGCG (1500 mg/kg, i.e., for 7 days) had a 138-fold rise in plasma alanine aminotransferase (ALT), according to a 2010 in vivo investigation (Lambert2010., et al).[(19)]In another study conducted in *vitro* bovine thymus DNA EGCG initiated the 8-oxide formation, causing oxidative damage to DNA that is associated with mutations and cancer.[(20)]According to another study, EGCG (20, 40, and 80 µM, 10, 60, and 240

min) damaged DNA in Nalm6 cells and human lymphocytes in a dose-dependent way. The greatest concentration of EGCG that resulted in a 50% reduction in Nalm6 survival and a 25% decrease in human lymphocyte survival was reached at 100 μM.Hence their inconsiderate use for the purpose of weight management must be avoided. [21]

Toxicity of *C. sinensis* (US and EFSA)

Case series and a systematic review by the United States Pharmacopeia have raised the issue of the potential for GTE to cause hepatotoxicity. In a large prospective study of GTE in postmenopausal woman at risk for breast cancer, GTE was associated with ALT elevations in 6.7% of patients compared to 0.7% of controls. Liver injury typically arises within 1 to 6 months of starting the product but longer and shorter latencies (particularly with re-exposure) have been reported.Approximately 10% of the green tea extract is composed of catechins; of these, epigallocatechin-3-gallate (EGCG) is present in highest concentration. There is great variability in the concentration of green tea extract, EGCG and other components among marketed products, which may explain while some products have been implicated in hepatotoxicity. Exposure of rat hepatocytes to EGCG has been shown to induce mitochondrial toxicity and generation of reactive oxygen species. The close association of liver injury from green tea with the HLA allele B*35:01 suggests an immunologic etiology. European Food Safety Authority (EFSA) Compendium of botanicals' mentioned Hepatotoxicity and listed in the compendium

Material and Methods:

A. Collection of raw green tea: Dried leaves of *C. sinensis*were collected from M/S Mohan Trader's, Lahori gate, Old Delhi. The entire crude drug was characterized and identified.

B.Standardization of leaves of C. sinensis: The leaves of *C. sinensis* was standardized with the help of following parameters:

i. **Ash value:**Percentage total Ash value was calculated as:[22]

% Ash value = weight of ash / weight of crude drug taken X 100

ii. **Acid insoluble ash***:* Percentage acid insoluble ash value was calculated as:[23]

% Acid insoluble ash value = Weight of acid insoluble ash / Weight of crude drug taken X 100

iii. **Water soluble ash:** Percentage water soluble ash value was calculated as:[24]

% Water soluble ash value = Weight of water soluble ash / Weight of crude drug taken X 100

iv. **Loss on drying:** Loss on drying was calculated as:[25]

% Loss on drying = Loss of weight in sample / Weight of the sample X 100

v. **Extractive Value:** Dried green tea powder was weighed and macerated for 6 hours, shacked for 5 minutes and allowed to stand for 18 hours and then filtered. Filtrate was placed in flat bottom dish to evaporate to dryness on a water bath. The residue was dried at 105°C for 6 hours and allowed to cool in desiccator for 30 min. The final residue was weighed and calculated.[26]

C.**Preliminary test for phytochemical screening:** Phytochemical tests for Alkaloids, Glycosides, Flavonoids, Saponins, Tannins were performed for estimating the presence of various phyto-constituents.[26]

D. **Preparation of Lyophilised extract of green tea:**

The green tea leaves were collected, cleaned and shade dried completely. The extraction process was carried out in soxhlet apparatus with 100gm of dried tea leaves in 1L distilled water for 24 hours at 50 –60^{o}C.[27] Further, the extract was filtered and lyophilized.

E. Estimation of total phenolic contents
Presence of total phenolic contents was determined by folina–ciocalteu colorimetric analysis based on oxidation reduction reaction.[28]Standard solutions of Gallic acid were prepared and 1mg/ml of green tea extract solution was prepared in methanol. Standard curve was prepared. After 2 hours of incubation at room temperature the absorbance was measured at 765nm on UV-Visible spectroscopy.[29]

The absorbance of extract was also measured on the same way as above. In place of Gallic acid 0.1 ml of green tea extract solution was taken. Total phenolic content was calculated.

F. Estimation of flavonoid content

Presence of flavonoids was determined by aluminum chloride colorimetric analysis.[30] Standard solution of quercetin was prepared and 1mg/ml of sample solution of green tea extract was prepared in methanol. Standard curve was prepared. After 30 min of incubation at room temperature the absorbance was measured at 415nm on UV-Visible spectroscopy.

The absorbance of extract was also measured on the same way as above. In place of quercetin, 0.5 ml of green tea extract solution was taken. Total flavonoid content was calculated.

G. **TLC Analysis of green tea**
TLC plate was prepared and sprayed with anisaldehyde mixed with glacial acetic acid, methanol and conc. sulphuric acid.[31]A mixture of chloroform: acetone: formic acid (5: 4: 1) was used as mobile phase. Spots were observed under short wave and long wave ultraviolet light. Visualized spots were marked with needle and were measured and recorded from the center point of application and R_f value were calculated.

R_f value = distance traveled by the solute front/distance traveled by solvent front.

H.HPLC analysis of green tea extract

Green tea extract was prepared using 0.05% formic acid in methanol (70%). The mixture was sonicated for 90 min and then centrifuged at 5000 RPM for 10 min. Supernatant was collected in a glass vial and 7 mL of solvent was added to the sediment pellet. It was diluted to 1:5 with 0.05% formic acid in 70% of methanol before analysis. The HPLC system used was – SHIMADZU LC-2010C HT and the core shell column used was (RSLC) 120 C-18 column. 2.5% of acetonitrile in water *and* 0.1% Trifloroacetic acid (TFA) in acetonitrile was used as mobile phase. The flow rate of the pump was at 0.8ml/min, and injection volume should be 2.0µl. The absorbance was recorded at 280nm.[32]

I.Green tea effervescent tablet were evaluated:

A. Pre-compression studies
B. Compression of tablets
C. Optimization of tablets

A.Pre- compression studies

a) **Angle of repose:** Angle of repose (Ɵ) = $\tan^{-1}$ (2h/d).[33]

(Ɵ) = $\tan^{-1}$ h/r

b) **Bulk density and Tapped Density:**Following formula for bulk and tapped density was used.[17]
Bulk density = initial mass/initial volume

Tapped density = initial mass/final volume

c) P**ercentage of compressibility index** was calculated as:[34]

C = tapped density –bulk density/ tapped density X 100

d) **Hausner ratio:** The following formula is used for calculating Hausner's ratio.[35]

H= tapped density/bulk density

B. Compression of tablets
Direct Compression method: The direct compression technique was used for the compression of green tea effervescent tablets.[36] Formulation were coded with the various codes as: F1, F2, F3, F4, F5, F6, F7 and F8. Briefly lyophilized green tea powder was passed through the sieve of size #18. All the excipients were properly weighed and passed through the sieve of size #25. Tablets were compressed under controlled temperature and humidity. After the compression, evaluation of the tablets was performed and the best result tablets were taken for strip packaging.

C. **Optimization of tablets:**
a) **Hardness: H**ardness tester was used for testing. [37]

b*)* **Friability:** 20 tablets were initially taken and weighed accurately and placed in the apparatus known as Roche Friability Test Apparatus. The percentage of friability was calculated using given formula. % Friability = Initial Wt –Final Wt / Initial Wt X 100. [38]

c) Weight variation: In order to pass the test weight variation should range between ±5% of the total weight.[38]

$PD = [(Wt_{avg} - Wt_{initial}) / (Wt_{avg})] \times 100$

Where, PD = Percentage Deviation

Wt_{avg} = Average weight of tablet

$Wt_{initial}$ = Individual weight of tablet

a) **Effervescence time:** Hot water was taken and tablet dip into it. The effervescence time was evaluated with the help of stopwatch. When the tablet was completely dissolved, Effervescence time was noted. The results given is in the result and discussion portion.

b) **Packaging of tablets:** Strip packaging was done at Abyss Pharma Pvt. Ltd. Mayapuri, New Delhi.

Result and Discussion

I. Standardization of leaves of C. sinensis:

1. **Physicochemical analysis of green tea leaves:**Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, loss on drying, extractive values were evaluated.

Table. No 3: Physicochemical analysis of green tea

Sr. No.	Parameters	Results%(w/w)
1.	Total Ash	7.78 (± 0.19)
2.	Acid insoluble ash	2.15 (±0.11)
3.	Water soluble ash	0.75 (±0.03)
4.	Loss on drying at 110°C	4.48 (±0.26)
5.	Extractive values (Distilled Water)	36.5 (±1.32)

2. Phytochemical screening of green tea leaves: Phytochemical tests for tannins, saponins, cardiac glycosides, terpenoids, flavanoids, alkaloids were evaluated.

Table No. 4: Preliminary phytochemical test for green tea

S. No.	Phytochemical test	Green tea
1.	Tannins	+ve
2.	Saponins	+ve
3.	Cardiac Glycosides	+ve
4.	Terpenoids	+ve
5.	Flavanoids	+ve
6.	Alkaloids	+ve

3.Total polyphenolic and flavanoid content in green tea sample: The total Phenolic content and flvanoid content was estimated by Folin-Ciocalteu reagent by UV-Visible analysis of green tea sample and Gallic acid was taken as standard.

Table No. 5: Polyphenolic and flavonoid content in the lyophilized powder of green tea

S. No.	Test	Result
1.	Total poly-phenolic content	452.8µg/ml
2.	Total flavonoid content	175µg/ml

5. **TLC Analysis of green tea:** Visualized spots were marked and were measured and recorded from the center point of application and R_f value were calculated.
Total no of spots found –6 spots

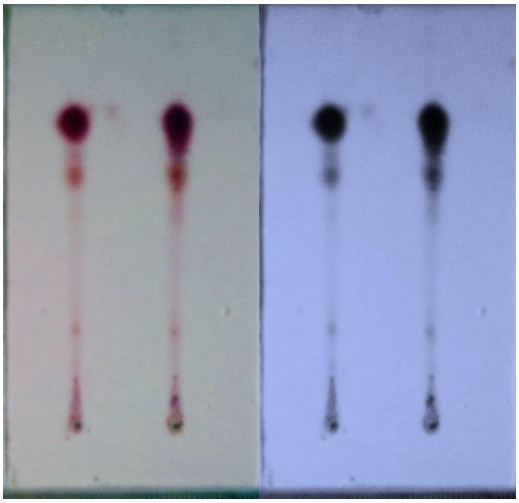


Fig.3: TLC plate of green tea Under normal light

Fig.4: TLC plate of green tea Under UV –light

6. **Rf value of Green Tea Components:** The best results were obtained using a mixture of chloroform: acetone: formic acid (5: 4: 1), which gave R_f values of 0.23 for EGCG, 0.27 for EGC, 0.32 for ECG, 0.51 for caffeine 0.56 unknown value and 0.64 for Gallic acid.

Table No. 6: Rf value showing the component of Green tea extract

S. No.	**R_f –Value**	**Chemical component**
1.	R_{f1}–2.4/10.6 = 0.23	EGCG
2.	R_{f2}–2.9/10.6 = 0.27	EGC
3.	R_{f3}–3.4/10.6 = 0.32	ECG
4.	R_{f4}–5.5/10.6 = 0.51	Caffeine
5.	R_{f5}–5.9/10.6 = 0.56	Unknown
6.	R_{f6}–6.8/10.6 = 0.64	Gallic acid

7. **Identification of Major Constituents:** Observation of HPLC analysis report showed the presence of polyphenolic component in green tea extract as:

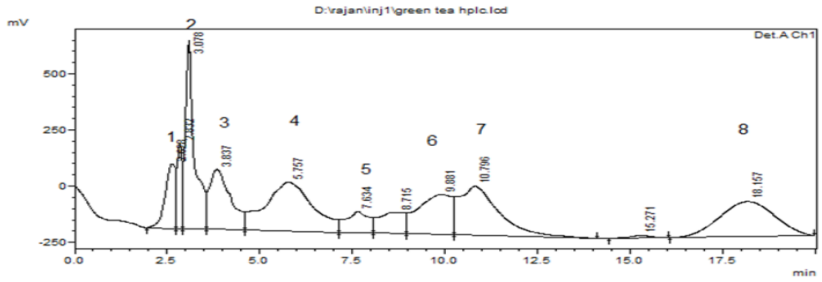
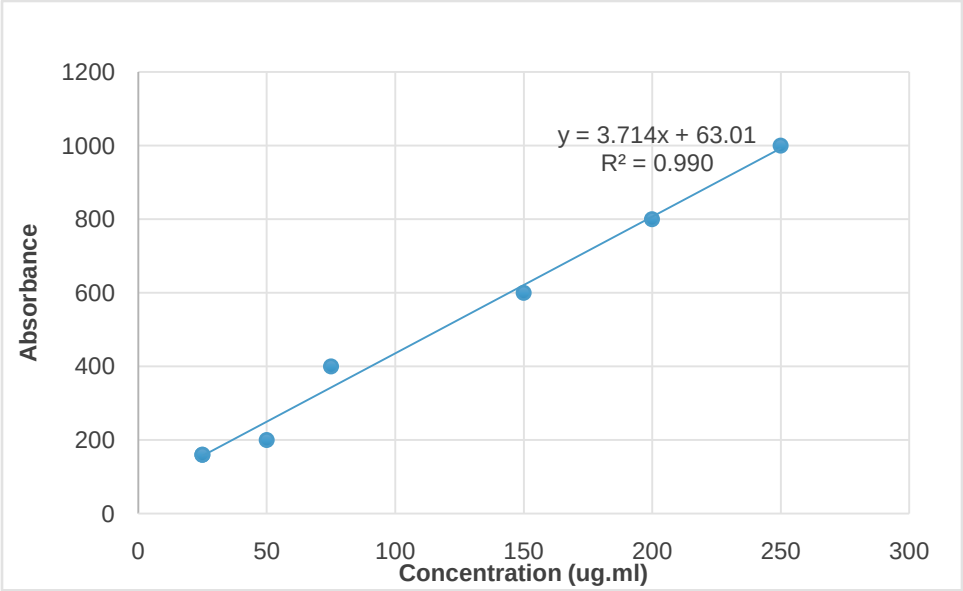


Fig. 5: Identification and Quantification of Major Constituents in Green Tea by HPLC



Graph 1: Standard Curve of epigallocatechin-3-gallate (EGCG)

Table No. 7: Components present in Green Tea extract

Peak no.	**Green tea component**
1.	Theobromine
2.	Gallic acid
3.	Gallocatechin
4.	Caffeine
5.	Catechin
6.	Epicatechin
7.	Epigalocatechin Gallate
8.	Epicatechin Gallate

8. **Pre-compression study:** The result of angle of repose, bulk density, tapped density, hausner's ratio, compressibility index etc are performed which are listed in below:

Table No.8: Pre-compression results of green tea formulation

S. No.	Tests	F1	F2	F3	F4	F5	F6	F7	F8
1.	Angle of repose(Ɵ)	26°	29.5°	28.2°	30.4°	27.9°	29.2°	30°	33.3°
2.	Bulk density (mg/ml)	632	640	688	708	720	731	735	742
3.	Tapped density (mg/ml)	744	782	826	848	840	862	880	897
4.	Hausner's ratio	1.17	1.22	1.20	1.19	1.16	1.18	1.20	1.21
5.	Carr's index	15.0	18.15	16.7	16.5	14.28	15.2	16.47	17.18
Flow property		good	fair	fair	Fair	good	fair	fair	fair

II. Optimization of formulation:

Total 8 types of formulation were developed out of which the best formulation was picked for further formulation of the tablets. All the ingredients were taken in percentage (% w/w).

Table No. 9: Green Tea effervescent tablet formulation (mg).

S. No.	Ingridients	F1	F2	F3	F4	F5	F6	F7	F8
1.	Green tea extract	62.3	55.7	50.4	46.0	42.3	39.1	36.4	34.1
2.	Citric acid	10.9	12.5	13.8	14.9	15.9	16.6	17.3	17.9
3.	Tartaric acid	5.6	5.8	7.0	7.6	8.0	8.4	8.7	9.0
4.	Sodium bicarbonate	19.0	20.5	25.4	27.8	29.8	31.5	33.0	34.2
5.	Sodium carbonate	1.56	2.0	2.8	3.2	3.6	3.9	4.2	4.4
6.	Sodium benzoate	0.16	0.15	0.13	0.12	0.11	0.10	0.09	0.08
7.	Stevia	0.46	0.41	0.38	0.35	0.32	0.29	0.27	0.25

III. **Evaluation of optimised formulation:** F5 was the best formulation with effervescent time of 29 seconds.

Table No.10: Evaluation of optimized formulation.

S. No.	Tests	F1	F2	F3	F4	F5	F6	F7	F8
1.	Hardness (kg/cm2)	1.5	1.6	1.8	2.1	2.5	2.8	2.9	3.3

2.	Friability %	0.4	0.25	0.3	0.12	0.1	0.1	0.12	0.1
3.	Weight variation (mg)	±4	±4.5	±2.3	±2.3	±1	±1.5	±1	±0.5
Effervescence time (sec)		52	54	40	38	29	35	42	56

IV. **Strip packaging with labeling:** The formulated tablets were taken to the strip packaging unit to Abyss Pharma Pvt. Ltd. New Delhi and were labeled.

Fig.6: Strip pack

Conclusion:

The primary objective of the present research work was to formulate effervescent tablets of green tea extract so as to deliver abundant health benefits along with improving patient compliance. Specialized techniques were employed for the preparation, standardization and optimization of test formulation. Physicochemical and phytochemical parameters were in favour of green tea. It is revealed that green tea used in the formulation was under great standards in terms of quality and hygiene. The formulation F5 passed all the evaluation test parameters with shortest effervescent time, better hardness and friability results. It was further observed that the amount of total polyphenolic content and the flavanoid content in lyophilized powder extract of green tea was 452.8µg/ml and 175µg/ml of sample respectively. TLC analysis gave R_f values of 0.23 for EGCG, 0.27 for EGC, 0.32 for ECG, 0.51 for caffeine 0.56 unknown value and 0.64 for Gallic acid. The HPLC analysis reported the presence of various peaks of theobromine, gallic acid, gallocatechin, caffeine, catechin, epicatechin, epigalocatechin gallate and epicatechin gallate. 160 green tea effervescent tablets were efficiently prepared and packaged under standard conditions. One tablet of our formulation can act as a substitute for one cup of green tea.

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