Development and Evaluation of Herbal Tablet Formulations of *Emblica officinalis* Gaertn. Leaf Extract

Abstract

In this study, a tablet dosage was formulated using a hydroalcoholic extract of the leaves of *E. officinalis* as the active ingredient. Herbal medicine has seen rapid growth in recent years, and these medications are gaining popularity in both developing and developed countries due to their natural origins and lack of side effects. The plant *E. officinalis* was collected, dried and extracted with alcohol: water (80:20 v/v). The dried hydroalcoholic extract was used for a single dose plasma study. Mechanical properties of tablets are critical tests that are frequently included in manufacturer's specifications and are quantifiable by the tablets' hardness, friability, and disintegration. The selection of excipients and procedures adopted to manufacture the dry extract obtained by hydroalcoholic extraction of *Emblica officinalis* Gaertn were found to be result-oriented as in is evidenced by the various investigation of the current research work.

Keywords: plasma study, *Emblica officinalis,* hydroalcoholic extract, Mechanical properties

INTRODUCTION

Herbal medicine has seen rapid growth in recent years, and these medications are gaining popularity in both developing and developed countries due to their natural origins and lack of side effects. Medicinal plants, minerals, and organic material are used in many traditional medicines. It is also reported by various literature *Emblica officinalis* Gaertn leaf extracts used as antioxidant also possesses various biological activities such as analgesic, anti-inflammatory, and antihyperglycemic activity. An extract of *E. officinalis* Gaertn used as an antioxidant and used in treatment of various Pharmacological activities such as anti inflammatory, analgesic and antihyperglycemic activities.[1] Amla is most extensively researched plant it includes biochemical compounds such as Phenols, alkaloids and juice contains highest proportion of vitamin C. Vitamin C is more efficient at preventing diabetes at a high dosage. It is reported to be effective in reducing the postprandial blood glucose levels, fasting blood glucose level and HbA1c levels.

Various scientists have reported antihyperglycemic agents and protects against complications. Ellagic acid in it is the effective α-glucosidase and α-amylase inhibitor.[2,3] Pharmaceutical dosage forms include excipients and pharmacologically active compounds that help in the development and production of tablet. The final dosage form's properties, particularly its bioavailability and stability, are highly dependent on the excipient selected, its concentration, and its association with both the active compound. To prepare a compressed tablet, an aqueous granulation technique was developed using approved excipients. In this study, a tablet dosage was formulated using a hydroalcoholic extract of the leaves of *E. officinalis* as the active ingredient.

MATERIALS AND METHODS

Material and Reagents

The plant *E. officinalis* was collected, dried and extracted with alcohol: water (80:20 v/v). The dried hydroalcoholic extract was used for a single dose plasma study. The solvents methanol, and acetonitrile from Qualigenes, orthophosphoric acid and dimethyl formamide (DMF) obtained from Rankem (RFCL Limited, Bangalore, India) of HPLC-grade, potassium dihydrogen orthophosphate from S. D. Fine-Chem (Ashwini Enterprises, Bangalore, India) of analytical reagent-grade were purchased and used. After filtration via an Ultipor N66 Nylon 66 membrane (0.45 m) P/N 60172 filter (Pall Life Sciences, Mumbai, India), all of the solutions were used, and water of ultrapure-grade of 18 MΩ-cm resistance was obtained using an Arium 611 UV purifier (Pall Life Sciences, Mumbai, India) (Sartorius Mechanotrics, Bengaluru, India). A suitable amount of *E. officinalis* extract was extracted with 50 percent aqueous ethanol and filtered after refluxing on a water bath at 100°C. The extraction was carried out twice more. The extraction solvents were mixed together, and the ethanol was extracted using low-pressure evaporation.

Formulation of Tablets

The compressible characteristics of the medication must be taken into account when choosing a tableting form. The most obvious and straightforward alternative will be to use wet granulation technique for medications that are poorly compressible and have moderate to high doses. The direct compression technique has many benefits for drugs with low to moderate doses such as since the substance is not expected to be exposed to moisture or heat, it is stable and provides consistency in results since tablets disintegrate directly, resulting in greater dissolution. In the

current analysis, however, wet granulation was used to prepare a traditional dosage type of *E. officinalis* extract.[4-8] **Table 1** describes the excipients profile.

Table 1. Excipients profile as per the specification and use.

S. No.	Excipients	Use
1	Lactose Monohydrate	Diluents
2	Starch	Disintegrant and Binder
3	Microcrystalline Cellulose	Adjuvant
4	Sodium Starch Glycolate	Super Disintegrant
5	Magnesium Stearate	Lubricant
6	Talc	Lubricant

Excipients Selection

Excipients are important in the design of a drug delivery system as it directly affects quality and efficiency. In the selection of excipients, the following points were considered: a) Compendial ingredients; b) Compatibility with each other; c) Stability; d) Drug release; and e) Availability. The selected excipient list used in the tablet dosage form along with their specification and test method is given below.[9-13]

Calculation of Dose

It is essential to calculate the expected dose of dried extract per tablet before finalizing the label claim. As 5 to 10 g of dried plant powder are used as a decoction in herbal remedies we considered 5 g of *E. officinalis* dried bark powder equivalent as a dose per day for the development of tablet dosage form. Therefore, 250 mg dried extract was taken per tablet. 3 tablets (represents 750 mg = 5 g leaves powder) may be carried as a daily dose. The equivalent ratio of *E. officinalis* Leaves / Hydroalcoholic extract / Gallic acid were used (**Table 2**).

Table 2. The equivalent ratio of *Emblica officinalis* Gaertn Leaves / Hydroalcoholic extract / Gallic acid.

Dried leaves Powder	Hydroalcoholic Extract	Content Gallic acid
100 g	15 g	1.5 g

5 g	0.75 g (750 mg)	0.075 g (7.5 mg)

*Leaves contain 12% Moisture

Content per Tablet: 250 mg of hydroalcoholic extract

Dose: 3tablets representing 750 mg of hydroalcoholc extract (e.g. 5g of dried leaves)

Granules

The dry powder extract and lactose were blended. Dry starch, sodium starch glycolate, and microcrystalline cellulose were individually passed through mesh no. 40 sieve and blended in a double cone blender for 15 minutes. The powder was mixed and blended for 5 min and was granulated with starch paste. The mass was passed through mesh number 12 sieves and dried at 500C ± 20°C in a hot air oven for 15 hrs. The dried granules were tested for LOD (NMT 4%), blended through a 16 mesh sieve, lubricated with a talc-magnesium stearate mixture, and compressed.[8]

Compression

The tablet compression machine (EMTECH®, India) single rotating 8punch was used for compressing the tablets. Theoretical average weight 750 mg were taken for compression into flat round tablets. The formulation for the tablet is given below (**Table 3**).

Table 3. Composition of the different batches of tablets.

Ingredients	B1 (in mg)	B2 (in mg)	B3 (in mg)	B4 (in mg)	B5 (in mg)	B6 (in mg)	B7 (in mg)	B8 (in mg)	B9 (in mg)
Extract	250	250	250	250	250	250	250	250	250
Lactose	327.2	337.2	317.2	317.2	327.2	307.2	307.2	317.2	297.2
Starch	34	34	34	34	34	34	34	34	34
Starch Paste	28	28	28	28	28	28	28	28	28
Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Sodium Starch	42	32	52	42	32	52	42	32	52

Glycolate									
Talc	34	34	34	34	34	34	34	34	34
Magnesium stearate	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
MCC	28	28	28	38	38	38	48	48	48
Total Weight	750	750	750	750	750	750	750	750	750

Evaluation

Drug Excipient Compatibility Study

To identify at the chemical interactions that could occur between the extract and the excipients. The Fourier-Transformed Infrared (FT-IR) spectra were captured using a Perkin Elmer® spectrophotometer (Model Spectrum 1, USA). A total of 2% (w/w) of the potassium bromide (KBr) sample was mixed with dry KBr and ground into a fine powder before being compressed into a KBr disc using a hydraulic press at 10,000 psi. The pellet that had been collected was scanned. The drug's IR spectra in KBr pellets were measured at a modest scanning speed of 4000-400 cm^{-1}.

Test for Disintegration

A digital disintegration testing apparatus (Electrolab, India) was used to calculate the disintegration times (DT) of the tablets in distilled water at 37±0.5°C.[8]

Dissolution Test

The tablets' *in-vitro* release was determined using a dissolution test apparatus (Electrolab®, India) with the paddle rotated at 100 rpm in 900 mL of 0.1 M HCl at 37±0.5°C. 5ml samples were withdrawn and replaced with fresh medium at fixed time intervals. The amount of drug released was determined spectrophotometrically at 314.8 nm.

Stability

The optimized batch B1 was subjected to three months of stability testing in accordance with ICH guidelines at a temperature of 30ºC ± 2ºC / 65% ± 5% RH and 40ºC ± 2ºC / 75% ± 5% RH.

Physicochemical parameters and Active content were considered for stability (ICH Harmonized Tripartite Guidelines, 2003)[14].

Optimization through Experimental Design

The 3^2 factorial designs were used to investigate the impact of variables. Hardness, disintegration time, and percent drug release (Y_1) were chosen as dependent variables, while SSG (X_1) and MCC (X_2) were held as independent variables.

Using statistical tools, the data analysis of values obtained from different batches for drug release and swelling is subjected to multiple regression analysis (Design Expert®, 8.0.7.1).[15] As shown below, the quadratic model was fitted using multiple regression analysis.

$$\mathbf{Y= \beta_0+ \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2}$$

Where X: levels of factors; Y: measured response; β: coefficient computed from the responses of the formulations. The levels of the two variables were chosen based on preliminary research conducted before the experimental design was implemented. Throughout the analysis, all other formulations and processing variables were kept constant. To classify the statistically significant terms, the coefficients corresponding quadratic effects (b11and b22) are calculated from the experiment results. Answer surface plots are then drawn using an equation containing only statistically significant terms to visualize the effect of changing variables at a glance (**Table 4**).

Table 4. Factor combination as per the experimental design.

Formulations	Coded value	
	X1	**X2**
B1	0	-1
B2	-1	-1
B3	+1	-1
B4	0	0
B5	-1	0
B6	+1	0
B7	0	+1
B8	-1	+1
B9	+1	+1

The layout and translation of coded levels in actual units in a 3^2-factorial experimental design are shown below (**Table 5**).

Independent variable: X1 SSG and X2 MCC

Dependent variable: Y1 (Hardness), Y2 (Disintegration), Y3 (% Drug release)

Table 5. Translation of experimental conditions into physical units for tablet.

Coded Levels	Actual Levels	
	X_1 (mg)	**X_2 (mg)**
-1	32	28
0	42	38
1	52	48

Acute oral toxicity study

The limit test according to OECD 423 was used to ensure that the chosen dose passed the toxicity test.[16,17] The key test is based on a single ordered dose progression in which each animal is dosed one at a time, with a minimum of 48 hrs between doses. The first animal is given a dose that is one step below the best LD_{50} estimate. The extract was finely powdered and suspended in 0.5% CMC in water and used the OECD guideline was followed for the procedure. The result was no death up to 2000 mg/kg of a mouse. 2000 mg / kg dose in mouse = 2000 × 3/27 = 222.2 mg / kg human dose. Hence, LD_{50} for human is more than 222.2 × 60 = 13332 mg / 60 kg average weight.

Acute oral toxicity tests were carried out in accordance with the OECD-423 guidelines (Acute toxic class method). In this analysis, Swiss (One sex) mice (n= 3/each dose) were chosen using a random sampling technique. The animals were fasted for four hours and only had access to water. The extract (suspended with 0.5 percent w/v, CMC) was given orally to different groups of mice at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg, and any behavioural changes were observed every hour for 24 hours, and then mortality was observed for three days.

Animal

The study used Swiss albino mice (one sex) weighing of 20-25g. Animals were fed a normal pellet diet (Pranav Agro Industries Ltd., Pune) and given free access to water while being held at a temperature of 24-28°C, relative humidity of 60-70%, and a 12-hour day-night period. Fasted animals were deprived of food for one hour but had unlimited access to water.

Anti-inflammatory activity

The plethysmographic measurement of carrageenan-induced acute rat paw edema caused by subplantar injection of carrageenan in the rat's hind paw was used to develop this procedure. The paw volume was measured using the method defined by Wilhmi and Domenjoz, which was later updated by Sirodia and Rao. Albino rats (Wistar strain) of either sex weighing 100-200 gm were used in this research, and they were divided into four classes, each with n = 6 animals. Group I served as the control and received Tween-80 (0.1 percent, 1 ml) solution orally, while group II received Diclofenac sodium at a dosage of 10 mg / kg body weight in Tween-80 (0.1 percent, 1 ml) and served as the normal. Finally, groups III and IV received orally the *E. officinalis* extract. The following formula was used to measure the percentage inhibition of paw edema in rats:

% Inhibition = (Control-Test) / Control × 100

Single-dose plasma study by HPLC

To identify the presence of gallic acid along with other phytoconstituents in plasma after the oral administration of a single dose of *E. officinalis* hydroalcoholic extract, we have optimized HPLC-UV based method. The optimized method was partially validated as per international guidance.[21] Waters® HPLC-LC2010A was equipped with an isocratic pump, autosampler, and SPD-M 10A vp PDA detector or SPD0AD vp UV detector (Kyoto®, Japan). CFR–21 part II software was used to collect data and conduct peak integration analysis. A Lichrospher 100 C18–ODS (octadecyl silane) (250 × 4.6 mm, 5 μm size) column (Merck, White House Station, NJ) was used to separate the samples. The temperature of the column was kept constant at 27°C. The injection volume was 20 micro litres, and the UV detection wavelength was 254 nm. The show lasted 15 minutes in total. The DLF vacuum filter pump and an analytical weighing balanceSartorius-BP-211D (Sartorius, Gottingen, Germany) were used (DLF Universal Limited, Delhi, India). For buffer solution preparation, the mobile phase was made by dissolving 1.36 g of potassium dihydrogen orthophosphate in 900mL of water and adjusting the pH with dilute orthophosphoric acid to 2.7 ± 0.2. Finally, water was added to bring the amount up to 1000 mL.

This buffer solution was combined with acetonitrile to make a 15:85 (v/v) final ratio. With an isocratic mode and a flow rate of 1 mL/min, a mobile phase of 0.01 M potassium dihydrogen orthophosphate (pH 2.7 ± 0.2)–acetonitrile (15:85, v/v) was used.

Preparation of gallic acid calibration curve

A gallic acid stock solution of 973.589 g/ml was prepared in a 10 mL volumetric flask by dissolving 10.089 mg of the compound of interest in 1 mL DMF and a small amount of HPLC grade methanol. The solution was then sonicated for 5 minutes, warmed on a steam water bath for 5 minutes, cooled, and HPLC methanol was added to make up to 10 mL. Calibration criteria at seven concentration levels were prepared. Calibration requirements for the analytical range 15-1000 g/mL were prepared by sequential dilutions of the stock solution with HPLC methanol to obtain 973.58 g/mL, 486.79 g/mL, 243.39 g/mL, 121.69 g/mL, 60.84 g/mL, 30.42 g/mL, and 15.21 g/mL. Each concentration was injected in triplicate with a volume of 20 L and chromate graphed under the conditions defined.

Preparation of plasma sample

E. officinalis hydroalcoholic extract in 0.50 % CMC suspension was orally administered to rats by the use of an oral catheter. After a designated period, the blood was collected through the retro-orbital plexus method. Rat plasma samples (0.5 mL) were precipitated with 1.3 mL acetonitrile containing 47.5 μg of p-nitrophenol. The supernatant was evaporated to dryness under nitrogen at 60°C after vortex mixing for 1 minute and centrifuging at 2500 rpm for 10 minutes. An aliquot (20 μL) was injected into the HPLC system after the residue was reconstituted with 0.1 mL of the mobile process.

Statistical Analysis

One-way analysis of variance was used in the statistical analysis, followed by the Dunnet's test. Significant P-values were described as those greater than 0.05.

RESULTS AND DISCUSSION

Drug Excipient Compatibility Study (FTIR)

The peak values (wave number) and the possibility of the functional group are recorded (**Figure 1**). The FTIR result shows (**Table 6**) that there is no significant change in the peak values of the

extract when tested individually or collectively. Hence, the ingredients selected as an excipient are compatible with the extract.

Table 6. Drug Excipient Compatibility.

PEAKS (cm^{-1})				Probable Functional Group	Normal Range (cm^{-1})
Extract	**Extract + MCC**	**Extract + SSG**	**Extract + Excipients**		
3408.86	3406.45	3419.0	3416.45	O-H stretch	3300-3600
2925.07	2921.45	2920.85	2920.24	C-H stretch	2700-3500
2139.94	2140.36	2131.23	2146.86	C=C	2100-2400
1611.72	1620.32	1616.20	1631.12	C=O	1600-1900
1426.22	1422.35	1412.23	1422.32	O-H Bending	1200-1500
1380.64	1375.54	1375.75	1378.23	C-H Bending	900-1400
1306	1304.54	1301.32	1312.21	C-H Bending	900-1400
1074.91	1075.23	1074.10	1068.10	C-O Stretch	900-1300

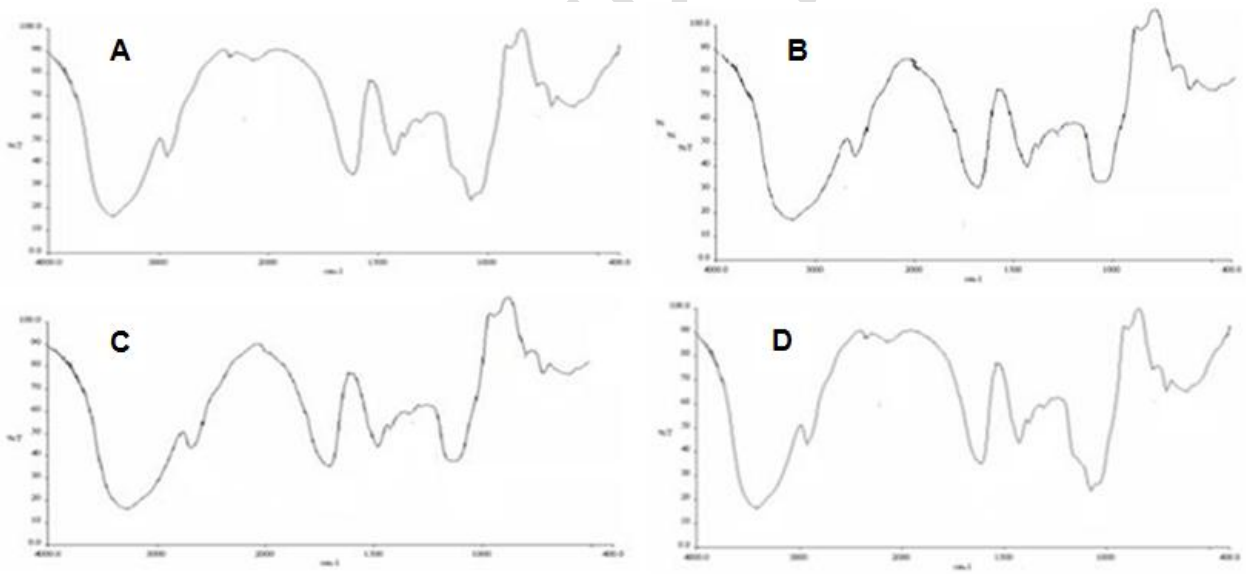

Figure 1. (A) pure *Emblica officinalis* Gaertn leaves extract; (B) pure *Emblica officinalis* Gaertn leaves extract + sodium starch glycolate; (C) pure *Emblica officinalis* Gaertn leaves extract + microcrystalline cellulose; and (D) pure *Emblica officinalis* Gaertn leaves extract + other usual excipients.

Physicochemical tests

All the batches of the formulated tablet were subjected to evaluation concerning Hardness (kg/cm^2), Thickness (mm), Diameter (cm), Friability (%), Disintegration Time (Sec), and Dissolution Time (%). The obtained result of the evaluation parameters are given below (**Table 7**). All the fabricated batches displayed the necessary hardness of more than 5 kg/cm^2along with friability value of less than 1%, ultimately representing the required strength and resistibility of the formulations. The tablet fabrication was done employing the punch to produce dimensions of 5 mm × 1.3 cm. The produced tablets were detected to be free from any problems like capping, picking, and chipping.

Table 7. Evaluation of the different batches of *Emblica officinalis* Gaertn leaves hydroalcoholic extract tablets.

Parameters	Specification	B1	B2	B3	B4	B5	B6	B7	B8	B9
Hardness (Kg/cm^2)	3-6	4	4	3	2.5	2.5	3.7	3.5	2	3
Thickness (mm)	5	5	5	5	5	5	5	5	5	5
Diameter (cm)	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Friability (%)	NMT 1.5%	0.4	1.32	1.3	1.64	1.75	1.45	1.53	1.6	1.4
Disintegration Time (Sec)	15-120	15	60	17	60	45	25	22	80	65
Dissolution Time (%)	NLT 90%	97.3	73.3	95.1	76.1	81.3	67.2	92.8	58.4	70. 5
Assay %	95±5	100	84	98	96	98	95	95	96	95

***In-vitro* release study**

It was revealed a % drug release (DR) of batch no B1; is 97.4%, batch no B2; 73.3%, batch no B3; 95.1%, batch no B4; 76.1%, batch no B5; 81.3%, batch no B 6; 67.2%, batch no B7; 92.8%, batch no B8; 58.4% and batch no 9; 70.5% at the end of 30 minutes. The sequence of drug release is shown below (**Figure 2**). The drug released from nine batches of formulations adopted zero-order kinetics. The release of drugs from tablets B1-B9 was investigated (**Table 8**).

Table 8. Drug release study of tablet batches.

Time (Min)	B1	B2	B3	B4	B5	B6	B7	B8	B9
5	43.1	23.9	39.9	31.8	32.8	32.6	33.9	23.9	30.1

10	54.3	38.2	51.3	42.7	48.6	40.3	46.7	28.6	39.6
15	60.5	46.7	58.7	52.2	56.1	46.4	59.2	35.7	48.5
20	75.6	53.7	73.3	61.5	63.4	53.7	69.6	42.9	53.6
25	90.2	61.4	87.6	69.3	74.1	58.6	82.4	50.1	61.2
30	97.4	73.3	95.1	76.1	81.3	67.2	92.8	58.4	70.5

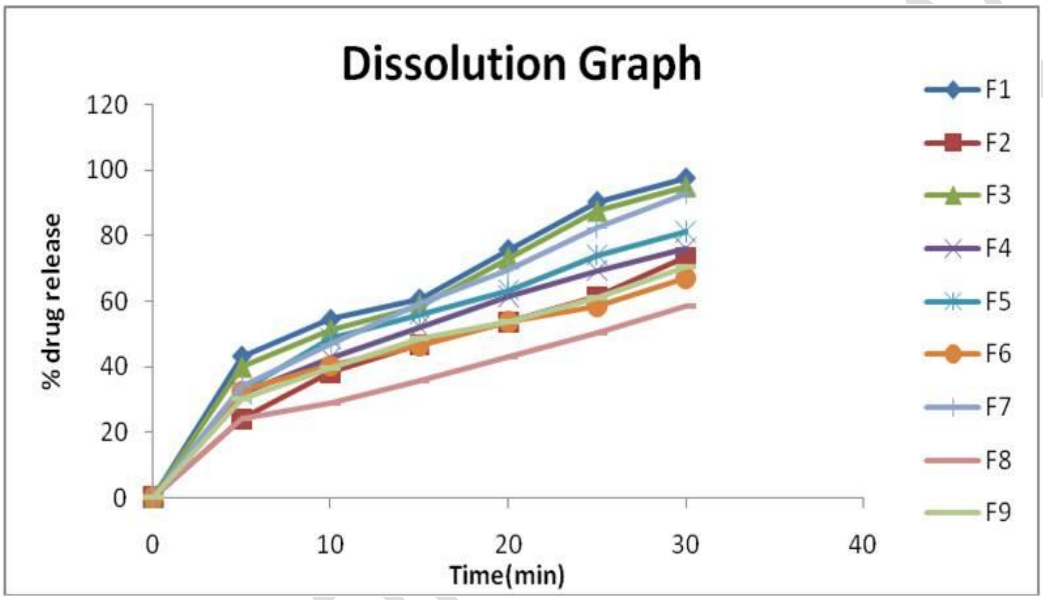

Figure 2. *In-vitro* drug release profile of tablets of *Emblica officinalis* Gaertn extract.

Stability studies

Sample tablets of the optimized formulation i.e. batch no: B1 was kept for stability studies. Stability studies for the tablet revealed good physical stability and organoleptic properties of tablets. Stability study observation of tablets Batch B1 is depicted below (**Table 9**).

Table 9. Stability study optimized Batch B1.

Duration (Days)	**Observations**							
	Weight (mg)	**Hardness (N)**	**Moisture (%)**	**Disintegration time (Sec)**	**Color**	**Odor**	**Stickiness**	**Active Content (%)**
30°C ± 2°C and 65% ± 5% RH								

Initial	750	4	2	30	Light Brown	Mixed odour of Aq. extract	No Stickiness	97.4
30	752	3.8	3	35	No Changes	No Changes	No Changes	96.8
60	749	4.1	3.2	35	No Changes	No Changes	No Changes	95.3
90	753	3.9	3.0	30	No Changes	No Changes	No Changes	96.8
40°C ± 2°C and 75% ± 5% RH								
Initial	750	4	2	30	Light Brown	Mixed odour of Aq. Extract	No Stickiness	97.4
30	752	3.9	3.1	35	No Changes	No Changes	No Changes	96.9
60	754	4.2	4	40	No Changes	No Changes	No Changes	96.3
90	748	3.8	3.4	35	No Changes	No Changes	No Changes	96.2

Optimization through Factorial Design

Optimization of formulation variables for preparation of *E. officinalis* tablet by using 3^2 Full factorial designs. The comparison of the experimental and expected values of the responses is performed to determine the model's reliability. To achieve the collection of objectives effectively, it was agreed to conduct a two-variable analysis at three experimental levels in the current study for the sake of simplicity. The *E. officinalis* tablet formulation method was thus subjected to a 3^2 complete Factorial Design. A total of nine batches of *E. officinalis* tablet were prepared and these batches were evaluated for hardness, disintegration time and % drug release of *E. officinalis*. Dependent variables' values of the formulations of *E. officinalis* tablets are explained (**Table 10**). These values are required in order for Design Expert software to generate polynomial equations for the dependent variable in question.

Table 10. Experimental runs and observed responses for 3^2 Factorial Design.

Batch	Code		Hardness (kg/cm^2) (Y1)	Disintegration time (sec) (Y2)	% Drug release (Y3)
	X1	X2			
B1	0	-1	4	20	97.4
B2	-1	-1	4	60	73.3
B3	+1	-1	3	17	95.1
B4	0	0	2.5	60	76.1
B5	-1	0	2.5	45	81.3
B6	+1	0	3.7	25	67.2
B7	0	+1	3.5	22	92.8
B8	-1	+1	2.0	80	58.4
B9	+1	+1	3	65	70.5

Response 1: Hardness

For Hardness, the following equation was obtained from the design model,

$$\mathbf{Y1 = 3.10 + 0.20X1 – 0.42X2 + 0.50X1X2 – 0.30X12 + 0.35X22}$$

The positive coefficient of X1 indicated an increase in Hardness with an increase in SSG, in the same way, the negative coefficient of X2 indicates a decrease in (Y1) i.e. Hardness with an increase in MCC concentration. 3.10 is the mean response i.e. mean hardness of tablet.

The surface responses plot for Hardness shows that SSG (X1) effect was Positive on Hardness. As the concentration of SSG increases (from -1 to +1) i.e. from 32-52 increase in Hardness.MCC also shows a negative effect on response Y1. As we increase the MCC concentration from 28-48 decrease in hardness but to a slight extent. Contour plot and Response surface plot showing the effect of factorial variables on Hardness is depicted below (**Figure 3**).

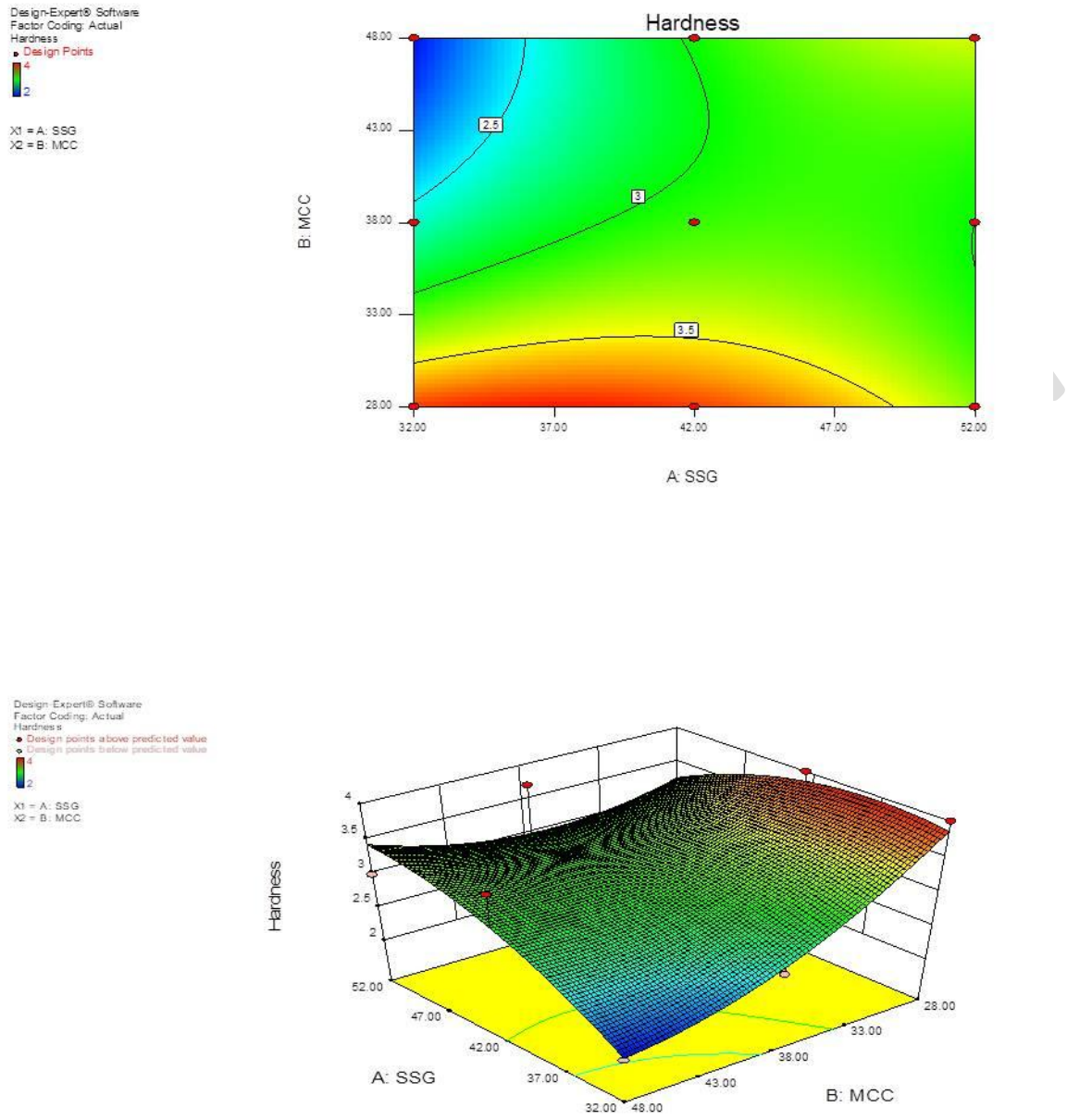

Figure 3. Contour plot and Response surface plot showing the effect of factorial variables on Hardness.

Response 2: Disintegration time

For Disintegration time, the following equation was obtained from the design model,

$$\mathbf{Y2 = 33.56 – 13.00\ X1 + 11.67\ X2 + 7.00\ X1X2 + 14.67\ X12 + 0.67\ X22}$$

The negative coefficient of X1 indicated a decrease in Disintegration time with an increase in SSG, in the same way, a positive coefficient of X2 indicates a decrease in (Y2) i.e. disintegration

time with an increase in MCC concentration. Also positive coefficient of the combination of both X1 and X2 indicates an increase in disintegration time with an increase in SSG and MCC. 33.56 is the mean response i.e. mean disintegration time of tablet.

Surface responses plot for disintegration time shows that SSG (X1) effect was negative on disintegration time, as the concentration of SSG increases from (-1 to +1) i.e. from 32-52 increase in disintegration. MCC shows a positive effect on response Y2, as we increase the MCC concentration from 28-48 increase in disintegration. The combination of SSG and MCC shows a positive effect on response Y2. The effect of factorial variables on disintegration time is depicted in the contour plot and response surface plot below (**Figure 4**).

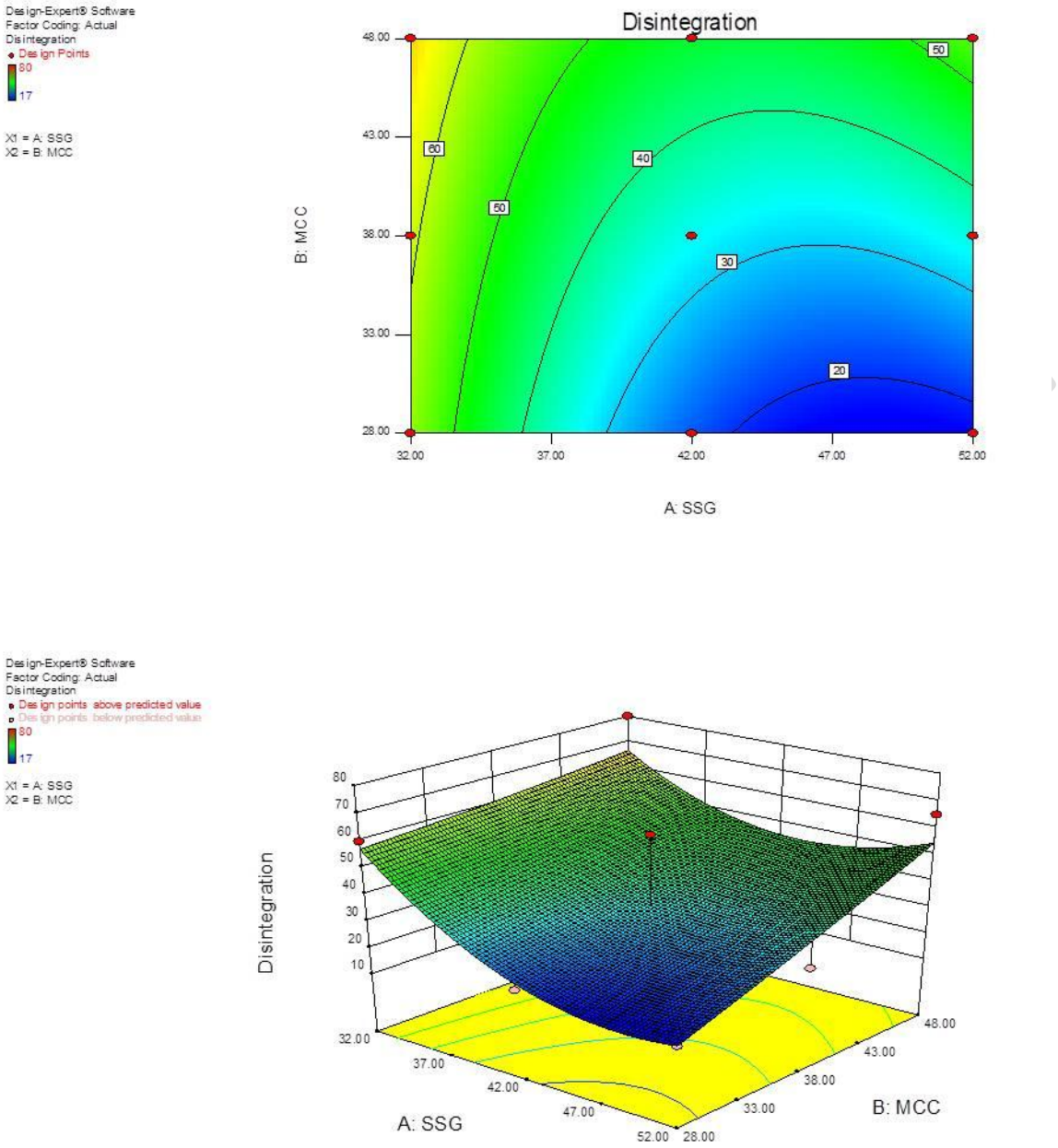

Figure 4. Contour Plot and Response Surface Plot showing the effect of factorial variables on Disintegration time.

Response 3: Percent drug release

For Percent drug release, the following equation was obtained from the design model,

$$Y2 = 84.51 + 3.30\,X1 - 7.35\,X2 - 2.43\,X1X2 - 14.47\,X12 + 6.38\,X22$$

The positive coefficient of X1 indicated an increase in % Drug release with an increase in SSG, in the same way, a negative coefficient of X2 indicates a decrease in (Y1) i.e. % Drug release with an increase in MCC concentration. 84.51 is the mean response i.e. mean % drug release from the tablet. Surface responses plot for % Drug release shows that SSG (X1) effect was positive on % drug release. As the concentration of SSG increases (from -1 to +1) i.e. from 32-52 increase in percent drug release. MCC shows a negative effect on response Y1. As we increase the MCC concentration from 28-48 decrease in % drug release but to slight extend. The effect of factorial variables on drug release is depicted in the contour plot and response surface plot below (**Figure 5**).

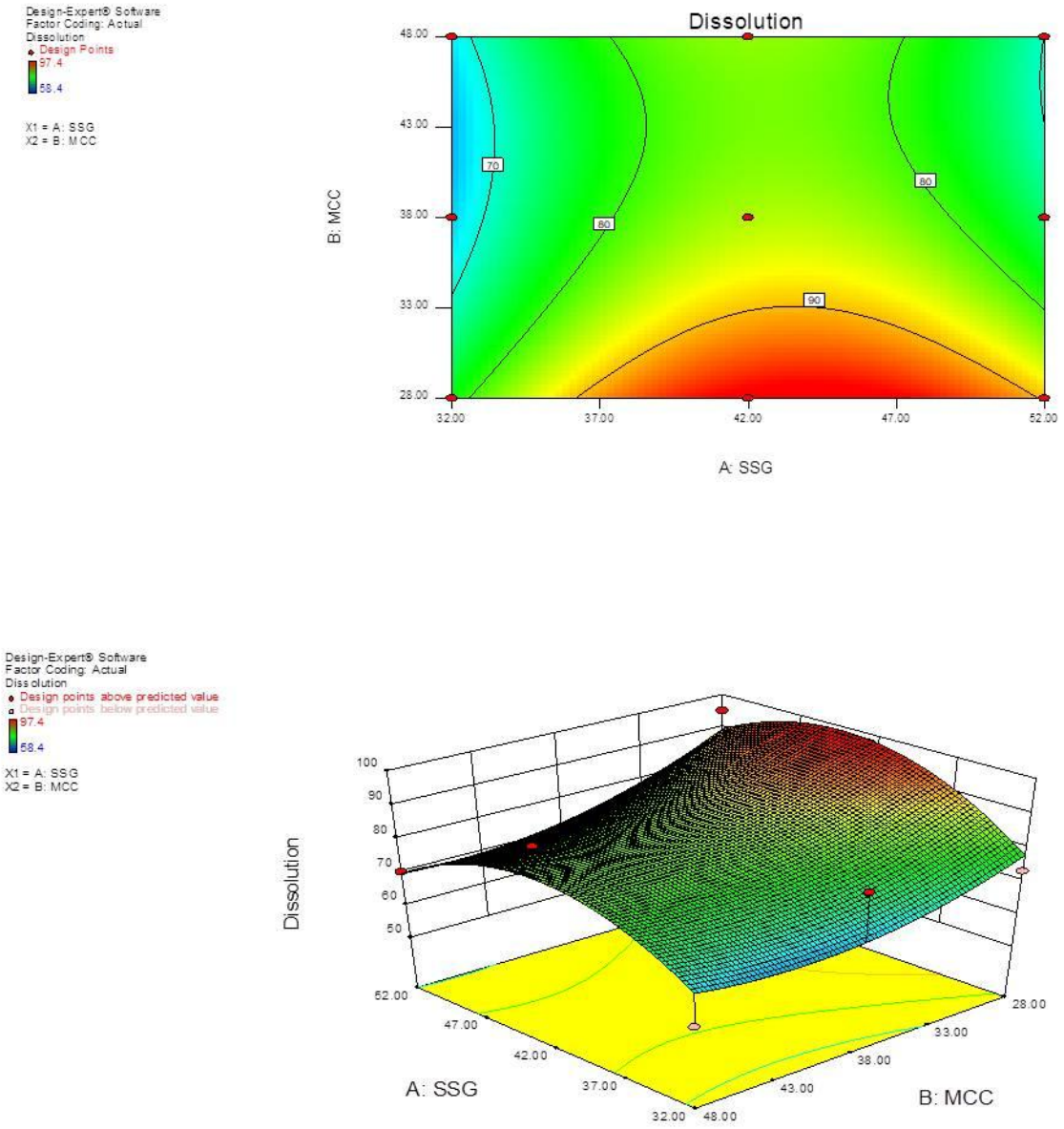

Figure 5. Contour Plot and Response surface plot showing the effect of factorial variables on Drug release.

Putting all these responses result & after setting goal "Six" solutions were obtained from nine combinations of categoric factor levels (**Table 11**). The best result was shown by the SSG at 43.97 mg & MCC at 28 mg concentration.

Table 11. Six Solutions from nine combinations of categorical factor levels.

S. No.	SSG	MCC	Hardness	Disintegration	Dissolution	Desirability
1	43.97	28.00	3.79575	19.1789	98.8108	0.953
2	44.08	28.00	3.79135	19.0323	98.8093	0.953
3	43.60	28.00	3.81103	19.7326	98.79	0.953
4	43.50	28.00	3.81491	19.8856	98.7776	0.953
5	40.50	28.00	3.90491	25.8856	97.0602	0.933
6	45.08	48.00	3.22044	45.4318	82.4418	0.591

Acute Toxicity Study

Acute oral toxicity tests were carried out in accordance with the OECD-423 guidelines (Acute toxic class method). Since there is no known dosage for the extract, it was subjected to an acute toxicity study using Swiss albino mice in accordance with OECD-423 guidelines, and it was found to be safe up to a dose level of 2000 mg/kg. The following are the general habits found within the first hour and after 24 hours of test drug administration (**Table 12**). According to the findings of the above acute toxicity report, there is no mortality after 72 hours and no improvement in the animal's general behavior.

Table 12. Acute Toxicity Study.

S. No.	Sign and Symptoms	OBSERVATION											
		5 mg/kg			50 mg/kg			300 mg/kg			2000 mg/kg		
		A1	A2	A3	A1	A2	A3	A1	A2	A3	A1	A2	A3
1	Tremor	-	-	-	-	-	-	-	-	-	-	-	-
2	Convulsion	-	-	-	-	-	-	-	-	-	-	-	-
3	Straub Reaction	-	-	-	-	-	-	-	-	-	-	-	-
4	Piloreaction	+	+	+	+	+	+	+	+	+	+	+	+
5	Catatonia	+	+	+	+	+	+	+	+	+	+	+	+
6	Loss of Righting reflux	-	-	-	-	-	-	-	-	-	-	-	-

7	Decreased motor activity	+	+	+	+	+	+	+	+	+	+	+	+
8	Increased motor activity	-	-	-	-	-	-	-	-	-	-	-	-
9	Sedation	+	+	+	+	+	+	+	+	+	+	+	+
10	Muscle Relaxation	+	+	+	+	+	+	+	+	+	+	+	+
11	Analgesia	-	-	-	-	-	-	-	-	-	-	-	-
12	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-
13	Lacrimation	+	+	+	+	+	+	+	+	+	+	+	+
14	Salivation	-	-	-	-	-	-	-	-	-	-	-	-

"-"Absent, "+" Present

Anti-inflammatory activity

Effect of *E. officinalis* leaves extract and tablet on carrageenan-induced paw edema indicating edema volume & edema inhibition is depicted below (**Table 13**). The paw edema inhibition effect comparison of the extract and equivalent quantity tablet (150 mg/kg) with standard drug diclofenac sodium 10 mg/kg body weight of the animal was found to be significant and comparable. This result establishes one of the uses of *E. officinalis* extract.

Table 13. Effect of *Emblica officinalis* Gaertn leaves extract and tablet on carrageenan-induced paw edema indicating edema volume and edema inhibition.

Group	**Dose**	**Paw volume after carrageenan injection**					
		1 hr		**3 hr**		**5 hr**	
		EV	**EI**	**EV**	**EI**	**EV**	**EI**
Control	---	2.40±0.03	---	2.65±0.02	---	2.58±0.03	---
Diclofenac Sodium	10 mg/kg	1.32±0.03	45.00	0.79±0.03***	70.18	1.05±0.02*	59.30
Extract	150 mg/kg	1.48±0.06	38.33	0.81±0.03**	69.43	1.11±0.03*	56.97
Tablet	450 mg/kg	1.51±0.02	37.08	0.89±0.04 ***	66.41	1.17±0.03*	54.65

Values are expressed as mean ± SEM (n=6); *EV – Oedema volume EI – Oedema inhibition*; *Significant at p< 0.05, ** Highly significant at p<0.01, * Very highly significant at p<0.001**

Single-dose plasma study

The optimized method was thoroughly validated using the following parameter (**Table 14**). Single-dose plasma study: Detection of gallic acid and other compounds is depicted below.

Table 14. Different parameters for HPLC Determination.

PARAMETERS	RESULT
Calibration range (µg/mL)	15–1000
Regression equation (Y)*	Y = a + bC
Slope (b)	42,246.000
Standard deviation of the slope (Sb)	254.542
Relative standard deviation of the slope (%)	11.232
Confidence limit of the slope	102. 193
Intercept (a)	483,579.732
Standard deviation of the intercept (Sa)	52,734.394
Relative standard deviation of the slope (%)	0.580
Confidence limit of the intercept†	20,482.596
Correlation coefficient (r)	0.9994
Response factor	48,969.1861

Identification of gallic acid in plasma samples

For the detection of gallic acid in rat plasma samples, an in-house HPLC method was developed. HPLC chromatogram of blank plasma sample is given below. The chromatogram showed the absence of any peak in the blank plasma sample. The presence of gallic acid in study plasma samples was identified by comparing the retention time (tR) with the peak of the reference standard. The reference standard gallic acid peak was observed at tR of 5.8 mins. The peak of internal standard (IS) was found at tR of 11.57 min. Interestingly, there are three different peaks (peak 1, 2 and 3) that were observed in the study plasma samples collected at 0.5 hr and 5.5 hr

after oral administration of the extract (**Figure 6**). This result has demonstrated that gallic acid could be one of the bioactive compounds for the activity of the gallic acid extract. Other components could have a role in gallic acid activity as well and needs further investigation.

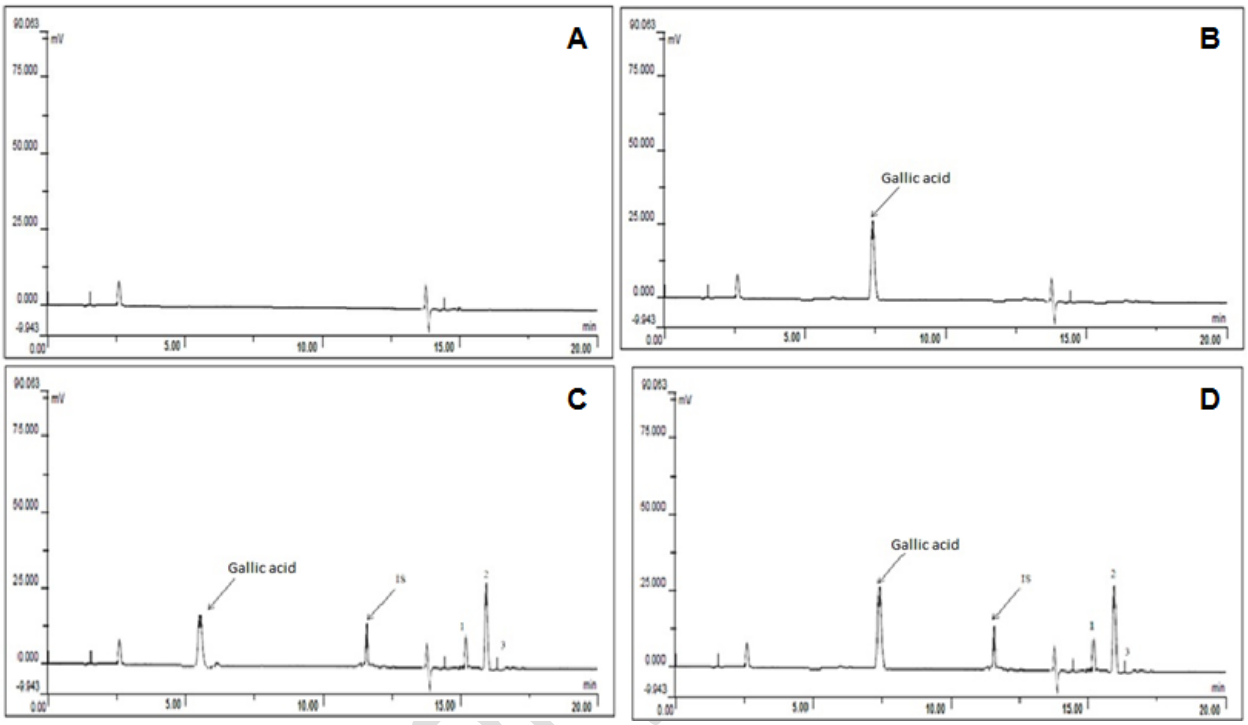

Figure 6. (A) HPLC-UV chromatogram of a blank plasma sample; (B) HPLC-UV chromatogram of the reference standard of gallic acid spiked in the blank plasma sample; (C) HPLC-UV chromatograms showing the presence of gallic acid at a retention time of 5.8 min with other peaks in the plasma samples collected after 0.5 hr of oral administration of extracts; and (D) HPLC-UV chromatograms showing the presence of gallic acid at a retention time of 5.8 min with other peaks in the plasma samples collected after 5.5 hr of oral administration of extracts.

DISCUSSION

Mechanical properties of tablets are critical tests that are frequently included in manufacturer's specifications and are quantifiable by the tablets' hardness, friability, and disintegration. The hardness of the tablet is a measure of its resilience, while the friability is a measure of its weakness. Both of these parameters indicate the tablets' ability to withstand fracture and abrasion during manufacturing and subsequent use. The pharmacopoeia requirements for tablet hardness

are largely determined by the tablet's intended use, while friability is described as a loss of less than 1% of the tablet's weight. At the concentration used, tablets prepared by wet granulation with starch paste as a binder had appropriate hardness and friability values, indicating that the wet granulation method is suitable for the production of *E. officinalis* leaves extract tablets. The requirement was met by all formulations of *E. officinalis* leave hydroalcoholic extract tablets, and in all nine batches. Batch no. B1 is the best because it has the shortest disintegration time (15 seconds) and the best dissolution (97.4 percent). The stability analysis showed that the tablets have strong physical stability and organoleptic properties. The anti-inflammatory effect was found to be quite comparative.

CONCLUSION

The selection of excipients and procedures adopted to manufacture the dry extract obtained by hydroalcoholic extraction of Emblica officinalis Gaertn were found to be result-oriented as in is evidenced by the various investigation of the current research work. However, the present scholar recommends further exhaustive work on different dosage forms like liquid orals.

REFERENCES

1. Aziz N, Wal P, Wal A, Saxena M.S. Evaluation of a Polyherbal Powder for Treatment of Diabetes Mellitus. Indian J Pharm Sci. 2019;81(6):1070-1077
2. Dasaroju S, Gottumukkala KM. Current trends in the research of *Emblica officinalis* (Amla): A pharmacological perspective. Int J Pharm Sci Rev Res. 2014;24(2):150-59.
3. Ansari A, Shahriar SZ, Hassan M, Das SK, Rokeya B, Haque A. *Emblica officinalis* improves glycemic status and oxidative stress in STZ induced type 2 diabetic model rats. Asian Pac J Trop Med. 2014;21-25.
4. Rowe R, Sheskey P, and Quinn M E. Hand book of Pharmaceutical Excipients, 6th edition, Pharmaceutical Press, Grayslake USA, 2009;371-729.
5. Ansel, H, Allen L. Pharmaceutical dosage forms and drug delivery systems. 7th edition Lippincott, 2000;347-356.
6. Aulton, M. Pharmaceutics: The science of Dosage form, Churchill Livingstone, 1996;304.

7. Lachman L, Lieberman HA. The theory and practice of Industrial Pharmacy, 3rd edition, Varghese publishing house, Bombay, 1990;293-329.

8. Majekodunmi S , Adegoke O, Odeku O.A. Formulation of the extract of the stem bark of Alstonia boonei as tablet dosage forms. Tropical Journal of Pharmaceutical Research, 2008;7: 987-994.

9. Rowe R P. Sheskey, Quinn ME. Handbook of Pharmaceutical Excipients, The Pharmaceutical Press and the American Pharmacists Association, London, 2009;6 : 364-372.

10. Rowe R, Sheskey P, Quinn M E. Handbook of Pharmaceutical Excipients, The Pharmaceutical Press and the American Pharmacists Association, London, 2009;6 : 129-132.

11. Rowe R, P. Sheskey Quinn M E. Handbook of Pharmaceutical Excipients, The Pharmaceutical Press and the American Pharmacists Association, London, 2009;6 : 663-666.

12. Rowe R, Sheskey P, Quinn M E. Handbook of Pharmaceutical Excipients, The Pharmaceutical Press and the American Pharmacists Association, London, 2009;6 : 404-407.

13. Rowe R, Sheskey P, Quinn M E. Handbook of Pharmaceutical Excipients, The Pharmaceutical Press and the American Pharmacists Association, London, 2009;6 : 728-730.

14. ICH Harmonized Tripartite Guidelines. Stability Testing of New Drug Substances and Products. ICH Committee 2003;8 : 162-176.

15. Schwartz J, O'Connar R, Schnaare RL. Optimization Techniques in Pharmaceutical Formulation and Processing :in Babker G.S. and Rhodes C.T. editior. Modern Pharmaceutical formulation, 2005;4 : 607-626.

16. Ecobichon D. J., The basis of toxicology testing. RC press, New York, 1997;43-86.

17. OECD Guideline for testing of chemicals, 17th December 2001;423: 1-14.

18. Gupta S, Prakash J, Awor L, Joshi T. Velpandian, Sengupta S. Anti-inflammatory activity of topical nimesulide gel in various experimental models. Inflamm Res 1996;45 : 590-592.

19. Latha M, Latha K, Vagdevi H, Virupaxappa S. Antiinflammatory activity of *Mangifera indica* L. Var. *Rasapuri* Root extracts. Journal of Chemical and Pharmaceutical Research, 2012;4(1) : 333-336.

20. Mohamed T, Azeem A, Dilip C, Sankar C, Prasanth N, Duraisami R. Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris*. Asian Pacific Journal of Tropical Biomedicine, 2011;147-149.

21. Gowda N, Kumar P, Pangha S, Rajshree M. ICH Guidance in Practice: Validated Revese-Phase HPLC Method for the Determination of Active Mangiferin from Extracts of *Mangifera indica* Linn. Journal of Chromatograpic Science. 2010;48(2): 156-160.