Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Open Access Research Article

Formulation and Evaluation of Novel Herbal Formulations Incorporated with Amla Extract for Improved Stability

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ABSTRACT

The objective of the present study was to formulate effervescent and fast dispersible granules by incorporating the fruit extract of *Emblica officinalis* as an alternate of liquid herbal juices available in market. Amla juice was extracted manually and then subjected to preliminary phytochemical screening which indicates the presence of alkaloids, glycosides, flavonoids, carbohydrates, phenolic compounds, proteins and phytosterols. Lyophilized amla powder was used to formulate effervescent and fast dispersible granules which were further optimized on the basis of concentration of superdisintegrants and effervescent producing agents like croscarmellose sodium, sodium starch glycolate, sodium bicarbonate and citric acid. Powdered formulations were then evaluated on basis of their flow properties like angle of repose, bulk density, tapped density, carr's index, hausner's ratio, effervescent cessation time and disintegration time. Among all the effervescent formulations F_2 was found to be optimum as it was having least disintegration time of 22 seconds and showed excellent flow properties. In case of the fast dispersible formulations the optimum strength were shown by formulations F_9 having croscarmellose with least disintegration time of 52 seconds. Total phenolic content of fresh amla juice were found to be 8.94 mg GAE/100 gm and estimation of ascorbic acid and gallic acid in lyophilized amla powder and developed formulations was carried out by HPTLC. *In vitro* antioxidant activity of lyophilized amla powder was evaluated by DPPH radical scavenging assay. IC_{50} value of lyophilized amla powder was found to be 32 ± 0.25 ug/ml calculated in comparison to standard ascorbic acid possessing IC_{50} value of 25.80 ± 0.2 ug/ml. Results of present study reveals that developed formulations may serve as alternate product with better quality, consistency and stability in comparison to available herbal liquid formulations.

Keywords: Anti-oxidant, Dispersible granules, Effervescent granules, *Emblica officinalis*.

Article Info: Received 17 May 2019; Review Completed 23 June 2019; Accepted 27 June 2019; Available online 15 July 2019

Cite this article as:

Madhavi N, Kumar D, Naman S, Singh M, Singh PA, Bajwa N, Baldi A, Formulation and Evaluation of Novel Herbal Formulations Incorporated with Amla Extract for Improved Stability, Journal of Drug Delivery and Therapeutics. 2019; 9(4):212-221 **http://dx.doi.org/10.22270/jddt.v9i4.3029**

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1. INTRODUCTION

Herbal juice has been used since the dawn of civilization to maintain health and to treat diseases. Use of herbal extracts in conventional medicinal system since antiquity and well established safety profile has resulted in wider acceptance by people. Inspite of wider acceptability there are some drawbacks related to herbal juices like stability, non-uniformity in dose and portability etc so there is a need to design and develop a cost effective novel formulations, which give consistent product quality. The objective of the present study was to develop novel solid formulations by incorporating the fruit extract of amla as alternate of liquid herbal juices available in market. Fresh amla is so sour that most people find eating it almost intolerable so the portability and convenience of effervescent granules will dramatically increases patient compliance. *Emblica officinalis* commonly known as amla is considered as natural wonder drug. It is the richest source of vitamin C[1]. The drug also contains secondary metabolites *viz*. gallic acid, ellagic acid, 1-*O*-galloyl-beta-D-glucose, 6-di-*O*-galloyl-D-glucose, quercetin, chebulinic acid, chebulagic acid, corilagin together with isostrictinnin[2].

The seeds of the Indian amla contain fixed oil, essential oil and phosphatides. The drug also contains secondary metabolites *viz*. gallic acid, ellagic acid, 1-*O*-galloyl-beta-D-glucose, 6-di-*O*-galloyl-D-glucose, quercetin, chebulinic acid,

chebulagic acid, corilagin together with isostrictinnin[2]. The seeds of the Indian amla contain fixed oil, essential oil and phosphatides. The bark and leaves of amla are rich in tannins[3]. Traditionally it is used in treatment of dyspepsia, gout, ulcer, diarrhea, migraine[4]. It also possess antioxidant[5], antidiabetic[6], hepatoprotective, antitumor and anti-inflammatory activity[7].

2. MATERIALS AND METHOD

2.1 Plant material

Amla fruits were procured from the local market of Moga (Punjab, India). The diseased, bruised and spotted fruits were sorted out. Healthy fruits were thoroughly washed in running tap water to remove dust and other extraneous materials. Plant herbarium was prepared and deposited in the Department of Quality Assurance, ISF College of Pharmacy for future reference.

2.2 Preliminary study of amla

In the preliminary study, various parameters for macroscopic and physical evaluation and composition of amla fruits have been studied.

2.3 Preparation of amla juice

Fruits were immediately pressed to obtain juice using a small laboratory manual press after cleaning and removal of seeds. Fine paste of extracted juice was made by placing pieces in a mixing jar. This paste was then wrapped into a lean cloth and squeezed with hand to get desire volume of amla juice. Organoleptic characters and yield of extracted juice was observed.

2.4 Lyophilization of extracted amla juice

The juice was first freeze dried at -20ºC for 12 h in deep freezer then lyophilized for next 8 hours consecutively for 3 days until the extracts was converted into fine powder. Initial drying was carried out at -20ºC and final drying was carried out at -55 ºC[8]. The lyophilized powder was stored in an air tight container and kept in the desiccators until used. Organoleptic properties and yield of lyophilized amla juice was observed.

2.5 Phytochemical screening

The amla juice so obtained was subjected to preliminary phytochemical screening for the detection of various phytoconstituents. Various chemical reagents were used for the detection of alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and amino acids[9,10].

2.6 Formulation and optimization of effervescent granules

Herbal effervescent granules containing amla juice powder were prepared by wet granulation method. Specified quantities of citric acid (5-25%), tartaric acid (15 -35%) and sodium bicarbonate (20 -50%) were accurately weighed and triturated with lyophilized amla powder (5 gm) into fine particles. Sufficient quantity of binders was added along with 10% PVP. Ethanol and isopropyl alcohol (1:1) was added to make a damp mass. This mass was passed through sieve no. 10 to get granules[11,12]. Granules were dried in hot air oven at 40°C and packed in air tight container. Optimization of effervescent granules was carried out by varying concentration of all ingredients.

2.7 Formulation and optimization of fast dispersible granules

For the preparation of fast dispersible granules, the ingredients were mixed in a systematic manner. The superdisintegrants such as croscarmallose sodium, sodium starch glycolate and crospovidone in varying concentration (1-5%) were used to develop granules. The drug and excipients except lubricants were sieved through sieve no.10. Lubricants were passed through calibrated 100#mesh separately. The drug was then mixed with lubricants and passed through sieve no. 10 to prepare the granules. Wet granules were dried in hot air oven at 40ºC for 1 hour and stored in air tight container[13,14]. Optimization of fast dispersible granules was carried out by varying concentration of all ingredients.

2.8 Evaluation of developed formulations

The developed formulations were optimized and evaluated on the basis of their flow properties[15,16]. Parameters like angle of repose, bulk density, tapped density, carr's index, hausner's ratio, effervescent cessation time and dispersion time were evaluated and optimum formulation was selected[17,18].

2.8.1 Angle of repose

The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface. The granules were carefully pored through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius of the base of the conical pile was measured and angle of repose (Θ) was calculated by using the following formula:

$$\text{Tan } \theta = h/r,$$

Where, θ = Angle of repose, h = Height of the cone, r = Radius of the cone base.

2.8.2 Bulk density

Granules (15 g) were introduced into a dry 100 ml cylinder, without compacting. The powder was carefully leveled without compacting and the unsettled apparent volume, VO, was read. The bulk density was calculated by using the following formula:

$$\rho_b = M / Vo$$

Where, ρ_b = Apparent bulk density, M = Weight of sample, Vo = Apparent volume of powder.

2.8.3 Tapped density

After carrying out the procedure as given in the measurement of bulk density, the cylinder containing the sample was tapped 500 times initially followed by an additional taps of 750 times until difference between succeeding measurement is less than 2% and then tapped volume, V_f was measured, to the nearest graduated unit. The tapped density was calculated, in gm per ml, using the following formula:

$$\rho_{tap} = M / V_f$$

Where, ρ_{tap} = Tapped density, M = Weight of sample, V_f = Tapped volume.

2.8.4 Carr's index

These differences between the bulk and tapped densities are reflected in the Carr's Index, which is calculated using the following formulas:

$$\text{Compressibility Index} = [(\rho_{tap} - \rho_b) / \rho_{tap}] / \times 100$$

Where, ρ_b = Bulk density, ρ_{tap} = Tapped density.

2.8.5 Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated as

Hausner's Ratio = Tapped density (ρ_t) / Bulk density (ρ_b)

Where, ρ_t tapped density and ρ_b is bulk density.

2.8.6 Effervescent cessation time

One dose of effervescent granules (5g) was poured in beaker containing 100ml distilled water. Effervescent production was observed and effervescent cessation time was recorded for each formulation.

2.8.7 Disintegration time of fast dispersible

One dose of the dispersible granules (5g) was poured in a beaker containing 100 ml of distilled water and the time required for all the granules to completely disperse/disintegrate was recorded.

2.9 Preparation of lyophilized amla powder sample

One gm of amla juice powder was added to 10 ml of methanol and filtered. Volume was made upto 50 ml with methanol in volumetric flask.

2.10 Estimation of total phenolic content in lyophilized amla powder

Total phenolic contents in amla extract were determined by using Folin Ciocalteu's reagent according to the modified method of Singleton and Rossi (1965). One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Thereafter, 1.5 ml Folin Ciocalteu's reagent (1:10) was added to each test tube and allowed to incubate at 25± 2°C for 5 minutes. Four ml of 20% (w/v) Na_2CO_3 were added to each test tube, adjusted with distilled water up to the mark of 25 ml and agitated. The quantification of total phenolics was assessed on the basis of gallic acid standard curve[19,20]. The absorbance of the resulting blue color in test was measured at 765 nm after 30 min (n=3). The results were expressed as percentage w/w**.**

Total phenolic content (%w/w) = GAE×V×D×10^{-6}×100/W

GAE - Gallic acid equivalent (µg/ml)

V – Total volume of sample (ml)

D - Dilution factor

W - Sample weight (gm)

2.11 Estimation of ascorbic acid and Gallic acid

2.11.1 Chemical and solvents

Ascorbic acid and Gallic acid were purchased from Central Drug House Ltd. New Delhi, India. Toluene, ethyl acetate, methanol, formic acid of analytical grade and precoated TLC silica gel 60 F_{254} aluminium plates (20 x10 cm, 0.2 mm thick) were procured from E. Merck. Germany.

2.11.2 Instrumentation

A HPTLC system (Camag, Switzerland) equipped with a sample applicator Linomat-V fitted with a 100 µl syringe (Hamilton, Switzerland), TLC Scanner III, Wincats 4.02, integration software (Camag, Switzerland) was used for the analysis and documentation. Analysis was performed by using TLC precoated silica gel 60 F_{254} aluminium plates (20 x10 cm, 0.2 mm thick) as stationary phase. The linear ascending development was carried out in a twin trough glass chamber.

2.11.3 Preparation of test samples

About 10 mg of lyophilized amla juice powder was accurately weighed and transferred in a 20 ml volumetric flask containing 10 ml of methanol. The resultant solution was sonicated for 30 min at ultra sonicator. The solution filtered through 0.45 µ filter for the analytical purpose.

2.11.4 Preparation of standard (Ascorbic acid and Gallic acid)

A stock solution of 1mg/ml was prepared for the marker by dissolving accurately weighed 1 mg of standard (Ascorbic acid and Gallic acid) in 10 ml of methanol followed by sonication for 30 minutes at ultra sonicator.

2.11.5 Sample application

The samples were applied in the form of bands (8 mm width) with a Camag Linomat- V sample applicator on pre-coated silica gel TLC plate. Application rate of 90nl/s was employed and space between the bands was 10 mm. Linear ascending development was carried out in a twin trough glass chamber presaturated with mobile phase. Densitometry scanning was performed on TLC scanner III in the absorbance/reflectance mode.

2.11.6 Estimation of ascorbic acid and gallic acid in lyophilized amla powder and in prepared formulation

10 µl of test sample was applied on TLC plate with the help of sample applicator Linomat V and used for the quantification. The amount of ascorbic acid and gallic acid was determined by developing the chromatogram (10 µL spot−1) in triplicate by maintaining the chromatographic conditions[21-23]. Stationary Phase**:** Pre-coated silica gel aluminium plate 60, Mobile phase**:** Toulene: ethylacetate: methanol: formic acid (2:4:4:0.05 v/v/v), Sample: Test sample (10 µl), Standard: Ascorbic acid 1 mg/ml [5 µL], Gallic acid 1 mg/ml [5µL], Prechromatographic derivatization: 0.02 M Sodium acetate solution (w/v) Scanning wavelength: 488 nm.

2.12 In vitro Evaluation of antioxidant activity

The free radical scavenging activity of the fresh amla juice and lyophilized amla juice was determined by using *in vitro* assays such as DPPH assay.

2.12.1 DPPH radical scavenging assay and determination of IC_{50}

The free radical scavenging activity of the fresh amla juice and lyophilized amla juice sample was determined by using *in vitro* assays *i.e.* DPPH assay[24]. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet color in the form of IC_{50} values.

2.12.2 Sample preparation

10 mg of powdered juice was weighed separately and dissolved in 10 ml of methanol to get 1000 µg/ml stock solutions. Lower concentrations (50, 100, 150, 200, 250 µg/ml) were prepared by diluting serially with methanol for extract.

2.12.3 Standard preparation

10 mg of ascorbic acid was weighed accurately and dissolved in 10 ml of methanol to get 1000 µg/ml stock solutions. Lower concentrations (1, 2, 3, 4, 5 µg/ml) were prepared by diluting serially with methanol.

2.12.4 Free radical scavenging activity (DPPH)

The 0.1 mM solution of DPPH in methanol was freshly prepared. Different concentrations of fresh juice extract and

lyophilized sample (50, 100, 150, 200, 250 µg/ml) were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard control. Same procedure was followed to evaluate the scavenging activity of the lyophilized extracts[25-31]. All the tests were performed in triplicate and the scavenging activity of the extracts or standard was calculated as the percent inhibition of DPPH radical scavenged activity using the formula.

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of samples, and its fractions.

3. RESULTS AND DISCUSSIONS:

3.1 Preliminary study of amla

Protein content, mineral composition and moisture content were preliminary evaluated in amla fruits. Composition and observation of various parameters for macroscopic and physical evaluation of amla fruit are summarized in Table 1 and Table 2.

3.2 Organoleptic studies and yield

Organoleptic properties of extracted and lyophilized amla juice along with yield were recorded and summarized in Table 3.

3.3 Amla juice preparation and lyophilization

The amla juice was prepared and lyophilized successfully. The % yield of the juice and lyophilized powder were 700ml/kg and 5.7gm/100ml respectively.

Table 1. Composition of amla fruit pulp

Composition	Percentage
Moisture	81.1%
Protein	0.5%
Fat	0.1 %
Mineral matter	0.7%
Carbohydrate	14.2%
Fibre	3.4%
Calcium	0.05%
Phosphorus	0.02%
Iron	1.2 mg/100g
Vitamin C	600 mg/100g

Table 2. Macroscopic evaluation of amla fruits

Parameters	Observations
Fruit Shape	Globular
Size of the fruits	3.8 cm
Fruit weight	41.24 – 52.48 g
Fruit color	Greenish yellow
Special features	Segmented through six ridges
Pulp weight	91% of the fruit wt
Ph	3.1-3.4
Odour	Characteristic
Taste	Astringent and sour

Table 3. Organoleptic properties and yield of lyophilized and extracted amla juice

Organoleptic Property	Extracted Amla Juice	Lyophilized Powder
Color	Light green	Greenish yellow
Odor	Characteristics	Characteristics
Taste	Astringent, Sour	Astringent ,Sour
Yield	700 ml/Kg	8.5 gm (± 0.5) per 200 ml

3.4 Preliminary Phytochemical Screening

The amla juice obtained was subjected to investigation for various phytoconstituents. It revealed the presence of different phytoconstituents like alkaloids, carbohydrates, glycosides, phenolics, protein, amino acid and phytosterols as in Table 4.

Table 4. Phytochemical screening of fresh amla juice

Constituents test reagents	Observation
Alkaloids	
Hager's reagent	+
Wagner's reagent	+
Mayer's reagent	+
Carbohydrates	
Molisch's reagent	+
Fehling's reagent	+
Bendict's reagent	+
Glycosides	
Legal's test	+
Flavonoids	
Libermann test	+
Fixed oils	
Saponification test	-
Spot test	-
Proteins and amino acids	
Millon's reagent	+
Biuret test	+
Ninhydrin test	+
Phenolics	
$FeCl_3$ Solution	+
Gelatin test	+
Saponins	
Foam test	-
Phytosterols	
Libermann test	+
Salkowski test	+

3.5 Formulation development and optimization of effervescent granules

Herbal effervescent granules were prepared by wet granulation method. Citric acid (11.78g), tartaric acid (23.56g), sodium bicarbonate (40.05g) and amla powder (24.6g) were triturated into fine powder. Then sufficient alcohol was added to make a damp mass. This mass was passed through sieve no. 10 to get granules. Total six formulations of effervescent granules were developed as shown in Table 5.

Table 5. Formulations of effervescent granules

Ingredients (gm)	Formulations code (Batches)					
	F_1	F_2	F_3	F_4	F_5	F_6
Amla powder	5	5	5	5	5	5
Citric acid	1.1	2.3	4.1	4.8	5.8	6.6
Tartaric acid	4.8	4.7	3.5	4.6	5.2	7.0
Sodium bicarbonate	8.1	7.8	6.6	9.3	11.2	14
Ethanol:IPA	1:1	1:1	1:1	1:1	1:1	1:1
Binder (%) (q.s)	10	10	10	10	10	10

3.6 Formulation and optimization of fast dispersible granules

The super disintegrants (Croscarmallose sodium, sodium starch glycolate and crospovidone) in varying concentration (1-5%) were used to develop different formulations by wet granulation method as shown in Table 6-8.

Table 6. Formulation of fast dispersible granules using crospovidone

Ingredients (gm)	Formulations code (Batches)		
	F_1	F_2	F_3
Amla powder	5	5	5
Crospovidone	1	2	3
Microcrystalline cellulose	10	10	10
Lactose	20	20	20
Binder (q.s)	10%	10%	10%
Cardamom flavor	QS	QS	QS

Table 7. Formulation of fast dispersible granules using sodium starch glycolate

Ingredients (gm)	Formulations code (Batches)		
	F_4	F_5	F_6
Amla powder	5	5	5
Sodium starch glycolate	1	2	3
Microcrystalline cellulose	10	10	10
Lactose	20	20	20
Binder (q.s)	10%	10%	10%
Cardamom flavor	QS	QS	QS

Table 8. Formulation of fast dissolving granules using croscarmellose sodium

Ingredients (gm)	Formulations code (Batches)		
	F_7	F_8	F_9
Amla powder	5	5	5
Croscarmellose sodium	1	2	3
Microcrystalline cellulose	10	10	10
Lactose	20	20	20
Binder (q.s)	10%	10%	10%
Cardamom flavor	QS	QS	QS

3.7 Evaluation parameters of effervescent formulation

3.7.1 Flow ability

Bulk density, tapped density , angle of repose hausner's ratio and carr's index of the prepared formulations were evaluated for their flow properties. . The angle of repose of F_2 effervescent formulation was in the range of 24.5°, which indicated good flow of granules; the carr's index was found to be in the range of 9.6 indicating good compressibility of granules. Hausner's ratio was found to be in the range of 1.137-1.25 and indicative of good flow properties as shown in Table 9.

3.7.2 Effervescent time

Among all the formulations F_2 Effervescent formulation possess effervescent time of 22 sec and was considered as optimum. Effervescent time of all the formulations is summarized in Table 10.

Table 9. Evaluation parameters of effervescent granules

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Hausner's ratio	Carr's index
F_1	0.57	0.77	22.5	1.352	25.97
F_2	0.58	0.66	24.5	1.137	9.6
F_3	0.55	0.78	23.4	1.418	29.48
F_4	0.54	0.68	20.0	1.259	20.58
F_5	0.61	0.69	23.3	1.131	11.59
F_6	0.68	0.47	22.4	1.088	8.10

Table 10. Effervescent time of different formulations

Batches	Effervescent times (Sec)
F_1	30 sec
F_2	22 sec
F_3	38 sec
F_4	24 sec
F_5	23 sec
F_6	25 sec

3.7.3 Particle size analysis

The particle size analysis showed maximum retention and was in the size range 1.40-1.70 mm for all the formulations as shown in Table 11. The mean diameter of the granules was found to be 1.40 mm approximately shown in fig 1.

Table 11. Particle size analysis

Sieve used	Opening size (mm)	Wt. retained (gm)	% Retention
4	4.75	0.0	0
6	3.35	0.021	0.14
8	2.36	0.749	4.996
10	1.70	3.003	20.02
12	1.40	9.558	63.72
16	1.00	1.356	9.04
20	0.85	0.192	1.28

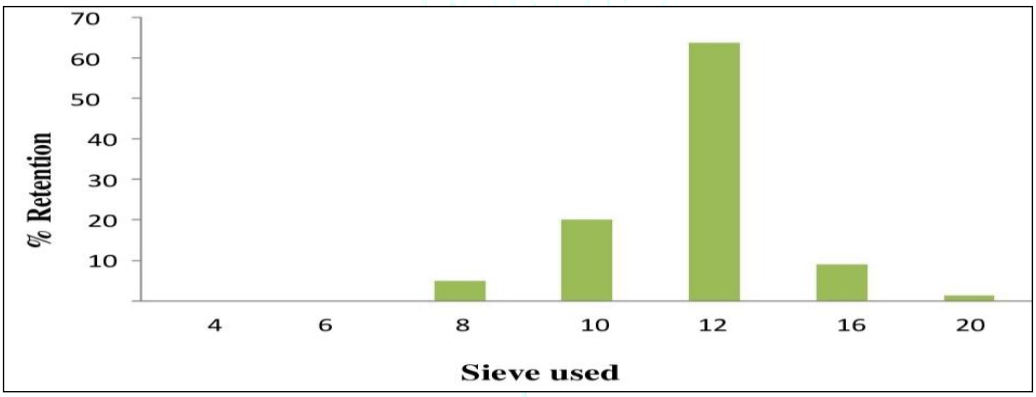

Fig 1. Graph plot of percentage retained

3. 8 Evaluation parameters of fast dispersible granules

Among fast dispersible granules formulations, having crosspovidone and sodium starch glycolate as super disintegrants exhibited long disintegration time, while in formulations having crosscarmellose showed fast disintegration as well as flow properties. Therefore, Crosscarmellose sodium was added as a super disintegrant. Formulation (F_9) having crosscarmellose as super disintegrant showed remarkable results having angle of repose (26.2 Θ), bulk and tapped density with value 0.54 and 0.71 respectively. On disintegration formulation F_9 shows least disintegration time as compared to other formulation. The results are summarized in Table 12, 13 and Fig 2.

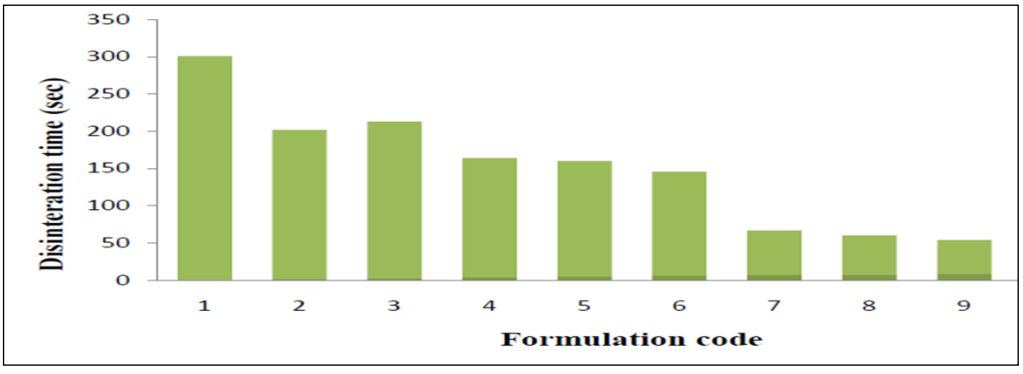

Fig. 2 Disintegration time of fast dispersible formulation

Table 12. Evaluation parameters of fast dispersible granules

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose
F_1	0.51	0.64	32.8
F_2	0.58	0.66	33.4
F_3	0.62	0.64	42.1
F_4	0.56	0.68	29.6
F_5	0.59	0.64	33.2
F_6	0.60	0.69	31.5
F_7	0.58	0.65	32.6
F_8	0.55	0.60	28.2
F_9	0.54	0.71	26.2

Table 13. Disintegration time of fast dispersible

Batches	Disintegration times (Sec)
F_1	303 sec
F_2	203 sec
F_3	210 sec
F_4	167 sec
F_5	160 sec
F_6	146 sec
F_7	73 sec
F_8	54 sec
F_9	52 sec

Table 14. % Inhibition by standard (ascorbic acid) in DPPH assay

Conc. (µg/ml)	% Inhibition (µg/ml)
100	75.5
50	62.08
25	52.41
12.5	46.56
6.25	43.82

Table 15. % Inhibition of DPPH by lyophilized amla juice

Conc. (ug/ml)	% Inhibition (ug/ml)
100	85.94
80	76.97
60	71.75
40	60.12
20	53.14

Table 16. IC_{50} value of standard, fresh amla juice samples in DPPH assay

Sample	IC_{50} (µg/ml) ± S.D.
Standard ascorbic acid	25.80±0.2 µg/ml
Fresh amla juice	32 ±0.25 µg/ml

3.9 Total phenolic content

Total phenolic content of fresh amla juice was estimated, gallic acid used as standard. The results were depicted as 8.94 mg GAE/100gm.

3.10 Estimation of ascorbic acid and gallic acid in prepared formulation.

Chromatogram of prepared formulations showed the presence of five peaks at deferent R_f, along with a peak each at R_f: 0.59 and 0.86 which were same for the standard ascorbic acid and gallic acid when applied in the same plate on different track. The chromatogram obtained from the optimized solvent system was illustrated in Fig.3

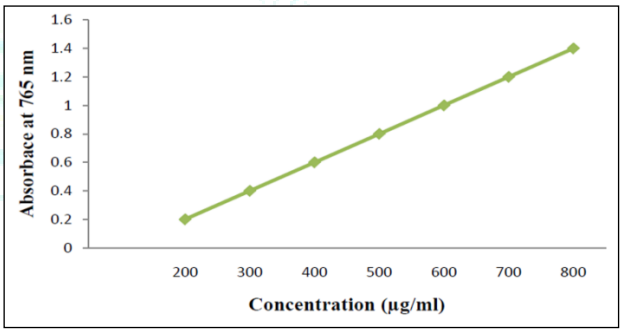

Fig. 3: Calibration curve of gallic acid for estimation of total phenolic content

3.11 Free radical scavenging activity (DPPH)

The lyophilized amla juice powder showed promising free radical scavenging effect in a concentration dependent manner. The extracted amla juice showed more scavenging activity as compared to reference standards. The reduction of DPPH by the extracted amla juice was very high and the scavenging ability increased with increasing concentration as compared to the standard. Ascorbic acid was used as reference standard for antioxidant activity. The results are depicted in Table 14, Table 15 and Table 16. The IC_{50} value for the extracted amla juice and lyophilized amla juice are represented in fig 4 and fig 5.

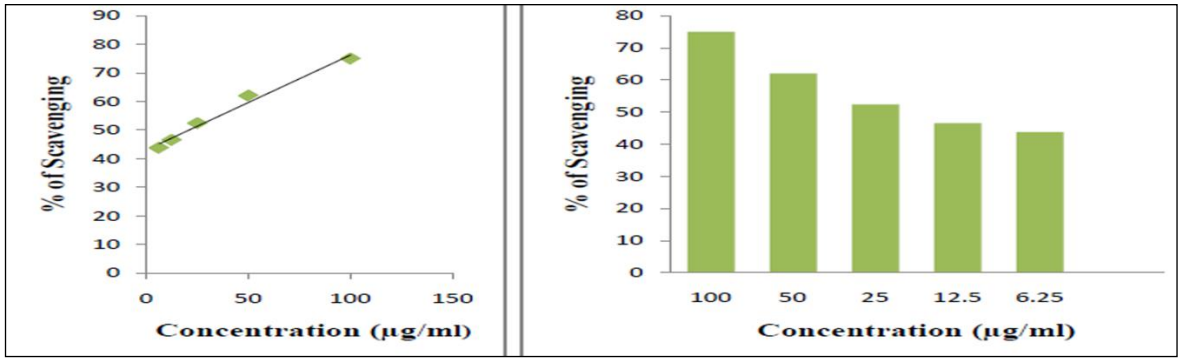

Fig. 4 Standard curve for IC_{50} determination using ascorbic acid.

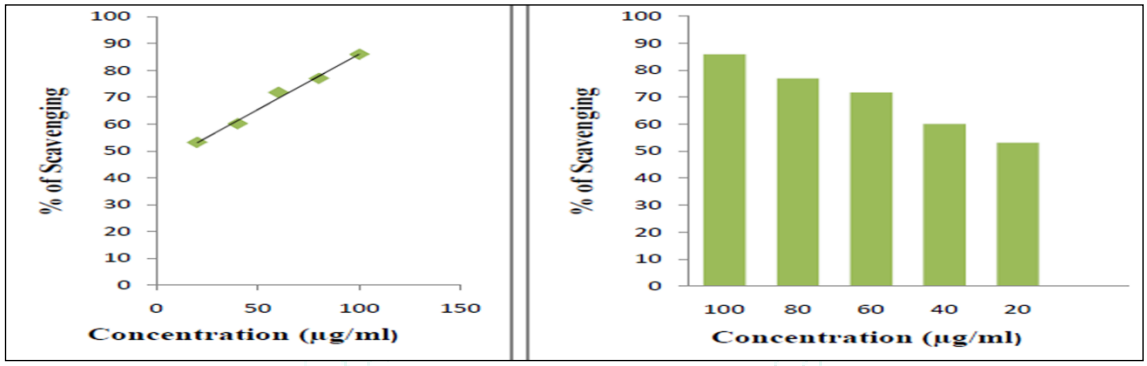

Fig. 5 IC_{50} determination for lyophilized amla juice

3.12 HPTLC Quantification and Estimation of Ascorbic acid and Gallic acid

3.12.1 Optimization of mobile phase

The optimized mobile phase containing Toluene: Ethyl acetate: Methanol: Formic acid (2:4:4:0.05 v/v/v) gave a sharp and well defined peak of ascorbic acid and gallic acid at R_f 0.59 and 0.86 after 20 minutes of chamber saturation. The chromatogram obtained from the optimized solvent system was illustrated in fig. 6.

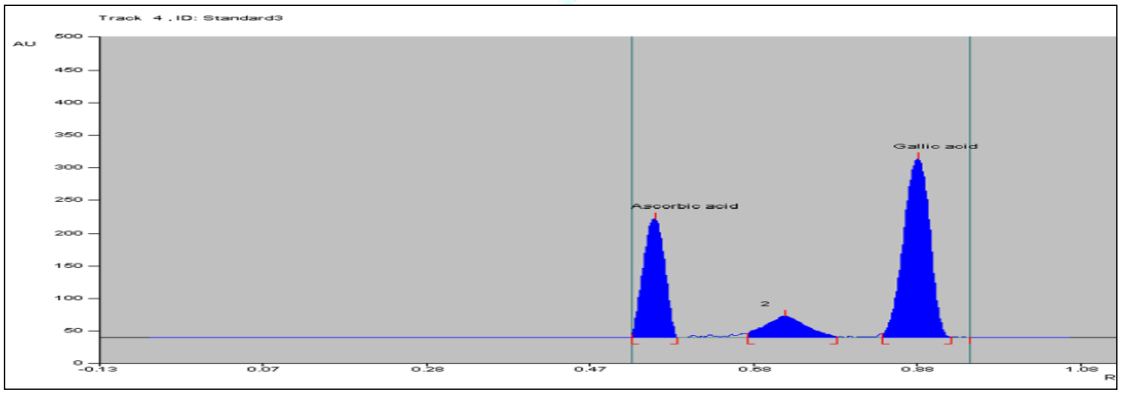

Fig. 6 Peak of standard ascorbic acid and gallic acid at R_f= 0.59 and 0.86

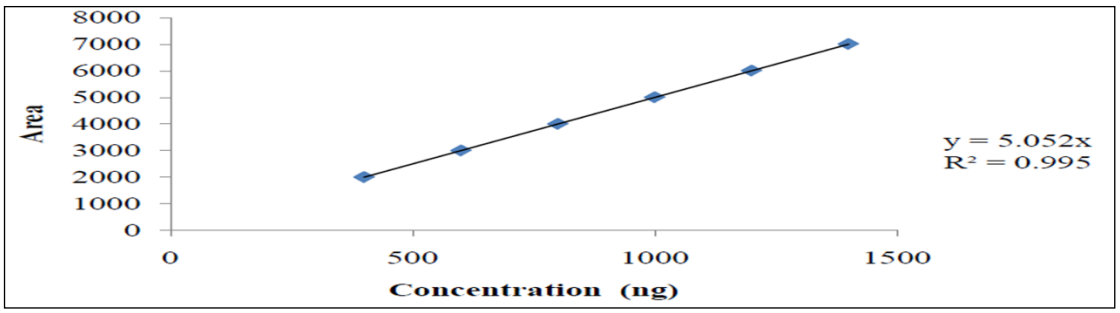

Fig. 7 Calibration curve of standard (ascorbic acid and gallic acid) over a range of concentration from 400 –1400 ng, r^2 = 0.9

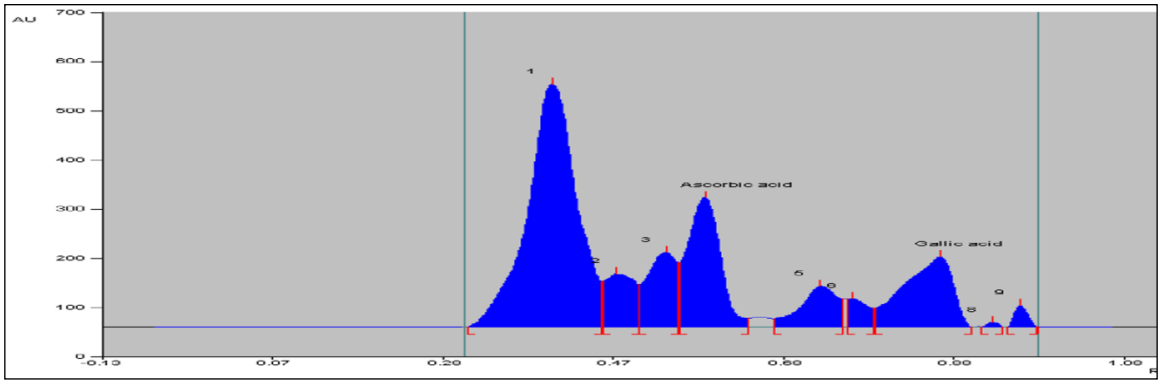

Fig. 8 Chromatogram of Lyophilized amla Powder

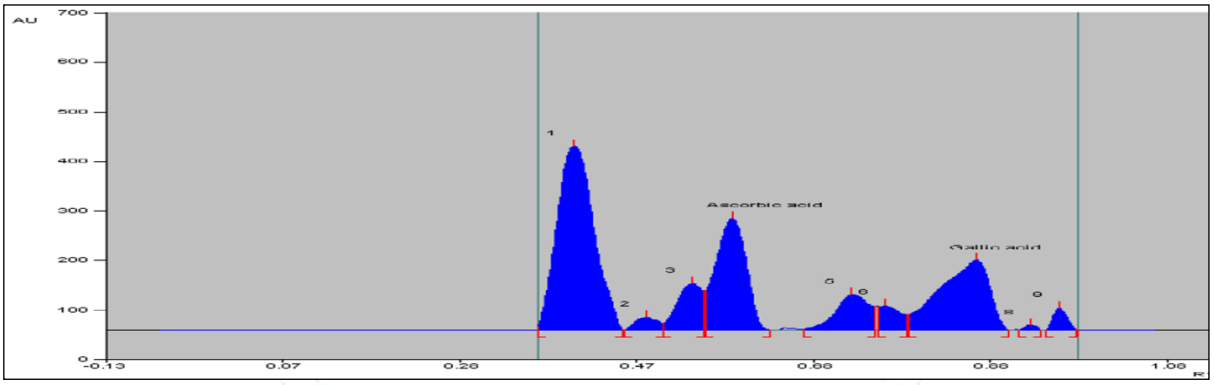

Fig. 9 Chromatogram of amla effervescent granules

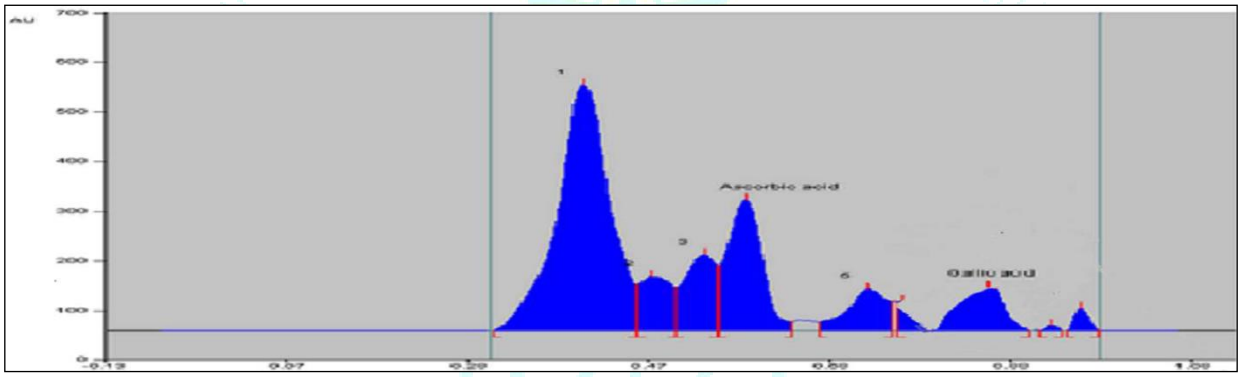

Fig. 10 Chromatogram of amla dispersible granules

3.13 Estimation of ascorbic acid and gallic acid in amla juice powder and formulations

Chromatogram of amla juice powder and formulations showed the presence of seven peaks at different R_f along with a peak at R_f 0.59 and 0.86 which were same for the standard ascorbic acid and gallic acid, when applied in the same plate on different track (Fig. 8-10). Spectra were found to be overlapped and the amount of ascorbic acid and gallic acid present in sample and formulations were evaluated.

CONCLUSION

The prepared formulations may be explored for development of a new range of herbal formulations with reproducible and stable characteristics. It is well documented that medicinal plants are natural gift to human lives to promote disease free healthy life. Amla is one of the precious gifts of nature to mankind. Hence it can be suggested to formulate effervescent and fast dispersible granules by incorporating the fruit extract of *Emblica officinalis* as an alternate of liquid herbal juices available in market. The effervescent formulations F_2 and fast dispersible formulations F_9 having croscarmellose as found to be optimum when evaluated on basis of their flow properties. Total phenolic content was determined and *in vitro* antioxidant activity was evaluated by DPPH assay. The content of ascorbic acid and gallic acid in lyophilized amla powder were estimated by HPTLC, so the portability and convenience of effervescent granules will dramatically increase patient compliance.

CONFLICT OF INTEREST

The author has no conflict of interest.

REFERENCES

1. Khosla S, Sharma S. A short description on pharmacogenetic properties of *Emblica officinalis*, Jour on Comp Med and Drug Discovery 2012; 2, 187-193.

2. Jain SK, Khurdiya D. Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of indian gooseberry (*Emblica officinalis* Gaertn.) Juice. Plant Foods for Human Nutrition, 2004;59, 63-66.3. Bajracharya MB. Ayurvedic Medicinal Plants and

General Treatment, Piyusavarsi Ausadhalaya Kathmandu, Nepal. 1979; 230.

4. Thakur R, Puri HS, Husain A. Major medicinal plants of India. Lucknow: Central Institute of Medicinal and Aromatic Plants. 1989; 585.

5. Ghosal S, Tripathi VK, Chauhan S. Active constituents of *Emblica officinalis*: Part 1- The chemistry and antioxidant effects of two hydrolysable tannins, Emblicanin A and B. Indian Jour of Chemistry. 1996; 35B, 941–948.

6. Devalaraja S, Jain S, Yadav H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. Food Res Inter, 2011; 44**,** 1856-1865.

7. Jeena KJ, Joy KL, Kuttan R. Effect of *Phyllanthus Emblica Phyllanthus amarus* and *Picrorhiza kurroa* on nitrosodiethylamine induced hepatocarcinogenesis. Cancer Lett,1999; 136:11–16.

8. Nireesha GR, Divya L, Sowmya C, Venkateshan N, Niranjan M, Lavakumar V. Lyophilization and freeze drying - An review, International Jour of Novel Trend in Pharm Sci. 2013; 3 (4) 87-98.

9. Kokate CK. Practical Pharmacognosy. 4thed. New Delhi: Vallabh Prakashan1994;pp.4.

10. Harborne JB. Phytochemical methods - A guide to modern technique of plant analysis, 3rded. UK: Champan and hall 1998; pp. 1-5.

11. Sandhya S, Gowthami G, Vinod KR, Sravanthi VE, Saikumar P, Rao KV, Banji D. Formulation and evaluation of herbal effervescent granules incorporated with *Limnophila indica* extract for bacillary dysentery, Ann of bio res, 2012; 3 (1):63-72.

12. Khalifa K, Ali M, Hassan SM, Abdalkreem M, Osman HM. Design formulation and evaluation of Senna effervescent tablets. Jour of For Pro & Ind, 2012; 1(2), 21- 25.

13 Lieberman HA, Lachman JB. Pharmaceutical dosage forms: tablets, Vol.1and 2, New york :Marcel dekker inc 1993**; pp.** 285.

14 Ansel HC, Allen V. Pharmaceutical dosage forms and Drug Delivery Systems, 6th ed. New Delhi: B. I. Waverly 1999; pp. 469-471.

15. James W. Pharmaceutical Preformulation: The physicochemical properties of drug substances: In: Aulton ME. Pharmaceutics the science of dosage form design, 2nd edition, Spain: Churchill Livingstone 2006; pp. 113-138.

16 Peter D, Gibson M. Oral solid dosage forms: Pharmaceutical preformulation and formulation. A practical guide from candidate drug selection to c ommercial dosage form, 2nd ed. New York: CRC Press 2008; pp.379-432.

17. Kulkarni U. Design and development of fast disintegrating tablets containing Amla powder by vacuum drying technique. Inter Jour of Pharm Res, 2001; 3**,** 2124-2134.

18.Kulkarni U, Manthale D, Patil BS. Design and development of fast disintegrating tablets containing Ashwagandha by sublimation technique. Inter Jour of Pharm Res, 2011; 64**,** 82-94.

.

19. Shanaz B, Arunachalam G, Jayaveera KN, Ashoka VL, Premakumari KB. Estimation of total phenolic content and in vitro antioxidant activity of *Barleria Montana*. Der Pharm Lett, 2011; 3 (4):178-182

20. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. Asian Pac Jour Trop Biomed. 2013; 3(8): 623–627.

21. Archana D, Rajasekaran A, Arivukkarasu R. HPTLC Method for estimation of Gallic acid and Rutin in Haritak An Ayurvedic Formulation. Inter Jour of Pharm Tech Res.2011; 3(2), 986-999.

22. Leela V, Saraswathy A. Quantification of Pharmacologically active markers Gallic acid, quercetin and lupeol from *Acacia leucophloea* wild flowers by HPTLC method . International Res Jour of Pharm. 2013; 4(2):146-150

23 Sajeeth CI, Manna PK, Manavalan R, Jolly CI. Quantitative estimation of gallic acid, rutin and quercetin in certain herbal plants by hptlc method. Der Chem Sin. 2010; 1 (2): 80-85

24. Jamuna, S, Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae). Jour of Appl Pharm Sci 2012; 2 (07):149-154

25. Baldi A, Hussain W, Tailor Y. Evaluation of in-vitro cultured cells of *Withania somnifera* for antioxidant activity. Curr trend in Biotech and Pharm, 2010; 4**,** 589-595.

26. Baldi A, Choudhary N. In vitro antioxidant and hepatoprotective potential of *Chenopodium album* extract. International Journal of Green Pharmacy, 2013; 7(1), 50-56.

27. Baldi A, Evaluation of in vitro antioxidant activity of *Eclipta alba*. Acta Horticulturae. 972, International Society for Horticultural Science 2013; pp.27-30.

28. Baldi A, Pandit MK, Ranka P. Amelioration of in-vivo Antioxidant Activity by Banana Extracts. International Journal of Pharmaceutical & Biological Archive, 2012;3(1), 157-161.

29. Baldi A, Hussain W, Tailor Y. Evaluation of in-vitro Cultured Cells of *Withania somnifera* for Antioxidant Activity. Current Trends in Biotechnology and Pharmacy, 2010; 4, 589- 595.

30. Singh PA, Brindavanam NB, Kimothi GP, Aeri V. Evaluation of in vivo anti-inflammatory and analgesic activity of *Dillenia indica* f. elongata (Miq.) Miq. and *Shorea robusta* stem bark extracts. Asian Pacific Journal of Tropical Disease, 2016; 6(1), 75-81.

31. Singh PA, Brindavanam NB, Kimothi GP, Verma R, Aeri V. A validated HPLC method for the determination of betulin in the stem bark of *Tectona grandis Linn.* International Journal of Pharmaceutical Sciences and Research, 2016; 7(2)719-723.

32. Singh PA, Vidhu A. Physico-chemical parameters and HPTLC fingerprinting profile of *Dillenia indica* Miq. f. Elongata (Miq.) and *Tectona grandis* Linn. with reference to betulin. International Journal of pharma and bio sciences, 2016;7(1)217-221