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FORMULATION AND EVALUATION OF EFFERVESCENT GRANULES PREPARED BY USING *MORINGA OLEIFERA* (LAM) LEAF EXTRACT

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ABSTRACT: *Moringa oleifera* leaves, have a high antioxidant content, which is beneficial for overcoming free radicals in the body. The leaves contents have the potential to be developed into a functional drink in the form of effervescent granules. The important aspects influencing the acceptability of granule is the type and ratio of the effervescent agent. In this study, citric acid and sodium bicarbonate were used as effervescent agents because they both provide acceptable texture and mouth feel. This study aimed to develop an effervescent granules formulation of *Moringa oleifera* leaf extract and to optimize the molar ratio of citricacid and sodium bicarbonate as the effervescent agent. Three formulations using different ratios of citric acid and sodium bicarbonate were selected, namely F1, F2 and F3. These formulations were developed using the wet granulation method. The results showed by effervescent granules of the three formulations exhibited good flow ability and good particle size distribution. The organoleptic and sensory evaluation results showed formulation F3 was the most acceptable in terms of colour, taste, aroma, and texture. In addition, *Moringa oleifera* extract showed IC_{50} values of 320µg/ml.

INTRODUCTION: Effervescent granules are popular delivery systems for many pharmaceutical products such as antacids, analgesics, and cough/cold formulations. They are fast dissolving, highly soluble, stable, convenient dosage forms [1]. The granules are added into a glass of water just before administration and the drug solution or dispersion is to be drunk immediately.

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The granules are quickly dispersed by internal liberation of Carbon dioxide in water due to interaction between acid with alkali metal carbonates or bicarbonates in the presence of water. Due to liberation in Carbon dioxide gas, the dissolution of the extract in water as well as taste masking effect is enhanced.

The advantages of effervescent granules compared with other oral dosage forms includes an opportunity for the formulator to improve taste, a gentler action on the patient's stomach and marketing aspects [2]. Many patients find it difficult to swallow tablets and hard gelatin capsules and thus do not comply with prescription, which results

in high incidence of non-compliance and ineffective therapy [3, 4, 5]. Granules show better flow ability, more stability, more wetting, and more uniformity in particle size, thereby drug content than powders [6]. In present work an attempt has been made to formulate a unit dose of effervescent granules containing immediate release of *Moringa oleifera* leaf extract using different ratio of acids and bases. The prepared granules were evaluated for flow rate, particle size, moisture content, pH and effervescence time of the granules.

Moringa oleifera is a potential source of antioxidants which is due to the presence of phenolics phytochemical compounds. Besides, other compounds which act as natural antioxidants are vitamin A, C, and E. The natural antioxidant content in dried weight basis of Moringa leaves includes 74-210 µmol/g for phenolics, 70-100µmol/g for vitamin C, 1.1-2.8 µmol/g for carotene, and 0.7 1.1 µmol/g for tocopherols (vitamin E) [7, 8, 9]. The antioxidants in the leaves are higher than other vegetables and fruits such as strawberries, carrots, and soybeans. Therefore, Moringa leaves are a source of natural antioxidants which are useful for counteracting free radicals and inhibiting cellular oxidation [10, 11]. One of the suitable preparation forms to be developed with the main content of *Moringa oleifera* leaf extract is effervescent granules.

MATERIALS AND METHODS:

Collection and Extraction of *Moringa oleifera* Leaves: *Moringa oleifera* leaves were obtained from Local Market of Cuddalore. It was later authenticated with the number 380/007/2022. All chemicals were obtained from Loba chemical Pvt Ltd, Mumbai. The fresh *Moringa oleifera* leaves were shade dried in the laboratory for 3 days. The stalks were removed and the leaves were size reduced using a mixer grinder. The weight of dried powder was noted. *Moringa oleifera* leaf powder was macerated in hot water for 24 hrs and temperature thermostatically maintained at 40°C and then filtered. The yield of the extract was determined by calculation, dividing the extract weight obtained by the weight of the powder [12].

Phyto-Chemical Screening: Active constituents in the extract of *Moringa oleifera* (Lam) was identified and detected by performing chemical tests. Phytochemicals such as tannins, phlobatannins, saponin, terpenoids such as flavonoids and alkaloids were detected based on standard tests.

Determination of Total Phenolic Content of *Moringa oleifera* Aqueous Extract: The test sample (*Moringa oleifera* aqueous extract) (500 µg/ml) was mixed with 0.5 ml of water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1: 1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 5 mL with distilled water. The reaction was kept in the dark for 30 min. and the absorbance of blue colour from different samples was measured at 765 nm.

Antioxidant Activity:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical Scavenging Assay: DPPH radical scavenging assay of formulation was modified method described by Perumal *et al.,* 2018. In brief, 0.135 mM DPPH was prepared in methanol. Different concentrations (5, 10, 20, 40, 80, 160 and 320 µg/mL) of sample was mixed with 2.5 mL of DPPH solution. The reaction mixture was vortexes thoroughly and kept at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability of extract to scavenge DPPH radical and control was calculated from the following formula,

$$\% \text{ DPPH inhibition} = (\text{Absorbance of control} - \text{Absorbance of test}) / (\text{Absorbance of control}) \times 100$$

The linear regression curve between % inhibition and sample concentration were calculated and vitamin C was used as a reference compound.

Formulation of *Moringa oleifera* Leaf Extract Effervescent Granules: The wet granulation method was used to prepare the moringa extract effervescent granules by varying the ratio of the acid –base compositions (1:2, 1:3). The formula and quantity of each component are shown in **Table 1**. The *Moringa oleifera* leaf extract was mixed with citric acid, and tartaric acid thoroughly. The mixture was sieved using sieve no. 12 to obtain uniform granules. The obtained granules were dried and added with sodium bicarbonate and others excipients.

Furthermore, both acid and base components were mixed, sieved through sieve no. 14, and dried in an oven at 50°C for 1 hr, to remove the water content [13, 14].

All the processes were performed; both formulations and physical properties of effervescent granules were performed in a special room with RH of 25% and temperature of 20-25°C

TABLE 1: FORMULATION OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT EFFERVESCENT GRANULES

Ingredients	F1(mg)	F2(mg)	F3(mg)
Moringa oleifera Leaf Extract	1500	1500	1500
Citric acid	500	400	750
Tartaric acid	50	50	150
Sodium bi carbonate	1100	1250	1400
PEG-600	50	50	50
Lactose	100	100	100
Flavouring agent	50	50	50
Aspartame	50	50	50
Total	3400	3450	4050

Physical Characteristics of Effervescent Granules:

Organoleptic Evaluation: *Moringa oleifera* leaf extract effervescent granules were organoleptically observed including shape, colour, smell and taste.

Evaluation of Flowability and Angle of Repose: Evaluation of flowability and angle of repose aims to predict the flow characteristics of the granules. This was determined using the funnel method [15]. The granules which weighed as much as 100 grams were put in a funnel with a closed bottom hole. At the time of measurement, the bottom hole was opened, while the time required for all the granules to flow down and form a heap was determined using a stopwatch.

The funnel method was conducted to measure the angle of repose. When granules samples are poured onto a horizontal plane, a conical pile will be formed. Furthermore, the angle of repose is the internal angle between the surface of the pile and the horizontal surface. The flow rate and the angle of repose of the sample were calculated as follows:

$$\text{Flow rate} = w/t$$

Where w is the weight (gram) and t is time (second).

While for the angle of repose:

$$\theta = \tan^{-1} h/r$$

Where h is the height of the granules forming the cone and r is the radius of the base.

Evaluation of Bulk Density, Tapped Density and Hausner Ratio: Measurement of bulk and tapped density was carried out by weighing 100 grams of granules and pouring them into a 250 mL measuring cup. The cup was tilted at a 45º angle when the granules were poured. Subsequently, the measuring cup was erected and shaken rapidly to even out the granule surface. The volume read was used to calculate the bulk density. The bulk density was calculated by the following formula.

$$\text{Bulk density} = Wo / Vo$$

Wo = granule weight

Vo = granule volume

The granules in the measuring cup were later tested for tapped density using a tapped density tester (Pharma Test D-63512 Hainburg, Germany). This was done at certain tapping intervals from 100 to 500 taps. Furthermore, the volume in the measuring cup was observed, until three consecutive observations showed a constant volume (Vt). Tapped density was calculated by the following formula.

$$\text{Tapped density} = Wo / Vt$$

Wo = granule weight

Vt = granule volume after compression.

The results of the bulk and tapped density were used to calculate the derived parameters of the granule flow characteristics, namely the Hausner

ratio. The Hausner ratio was calculated through the ratio between the tapped density and bulk density.

Evaluation of Granules Particle size Distribution: Evaluation of the granules particle size distribution was carried out with a standard sieve set. The sieves and collection pans were orderly arranged from top to bottom as follows, 20, 30, 50, 60, 80, 100, and collection pans. Furthermore, the sample used was 100 grams of effervescent granules.

At the initial stage, each sieve and collection pan were weighed, and the arrangement was placed on the "Retsch Vibrator. Subsequently, the granules were placed on the top sieve, closed, and tightened. A set of sieves was vibrated at a frequency of 60 Hz for 20 mins and weighed. Based on the data obtained, the weight of the granules contained in each sieve and the collection pan was determined.

Evaluation of the Granules Moisture Content: Moisture content evaluation was carried out by weighing 5 grams of the granules and placing them on the moisture content analyzer.

The instrument was run for 15 min with an annealing temperature of 100°C. The previous validation exhibited that for these granules 15 min had been reached a constant weight. The moisture content was indicated on the instrument in percentage.

RESULT AND DISCUSSION: Phytochemicals are the chemical constituents in plants with distinct physiological action on the human body. Alkaloids, flavonoids, phenolics, terpenoids, and essential oils are some of the important bioactive phytochemicals.

TABLE 2: RESULTS OF PHYTOCHEMICALS PRESENT IN *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT

Phytoconstituents	Aqueous Extract
Tannins	+
Terpenoids	+
Alkaloids	+
Reducing sugars	+
Flavanoids	+
Phlobatannins	–
Saponins	+
Anthraquinones	+

"+" indicates the presence of phytoconstituents. "" indicates the absence of phytoconstituents.

It is observed that the geographic location of the plant and the solvent system used in the extraction process may act as determining a factor for the distribution of these phytochemicals.

Phenolic Content of *Moringa oleifera* Leaf Extract: The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid (2-64 µg/ml), y = 0.0198x + 0.1427, R^2 = 0.9747.

TABLE 3: CONCENTRATION VS ABSORBANCE OF GALLIC ACID

Concentration µg/ml	Absorbance
2	0.1676
4	0.2953
8	0.3666
16	0.4823
32	0.8036
64	1.3783

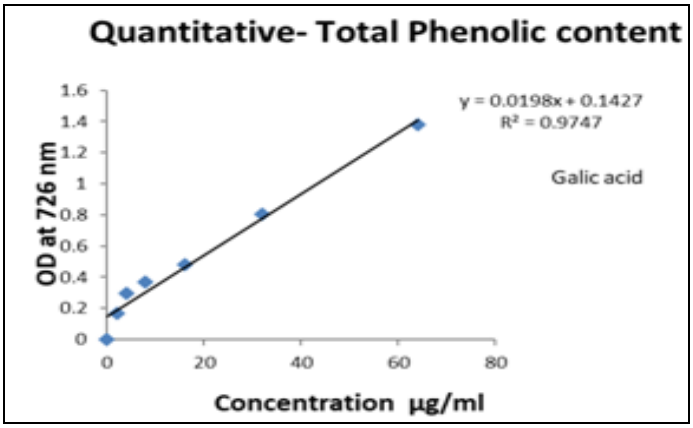

FIG. 1: STANDARD CURVE OF GALLIC ACID

Total phenol content in *Moringa oleifera* leaf extract using the calibration curve, was found to be 4.19 mg of Gallic acid equivalents/g dry weight of extract. The result shows that natural polyphenols of *Moringa oleifera* can remove free radicals, activate antioxidant enzymes, and inhibit oxidases**.**

Antioxidant Activity of *Moringa oleifera* Leaf Extract: The *Moringa oleifera* leaf extract inhibition percentage was 14.03% and for vitamin C was 93.76% and the corresponding IC50 values was 320µg/ml and 21.64µg/ml respectively.

However, these granules can remove free radicals due to the presence of Phenolic compounds. Therefore, *Moringa oleifera* leaf extract effervescent granules can be used as a food supplement to protect the human body from free radicals and inhibit oxidases.

TABLE 4: ANTIOXIDANT ACTIVITY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT

S. no.	Concentration µg/ml	% Inhibition of standard Ascorbic acid	%Inhibition of *Moringa oleifera* extract
1.	5	14.27±0.57	1.46±0,37
2.	10	31.09±0.51	4.30±0.52
3.	20	55.95±0.94	5.81±0.49
4.	40	70.79±0.42	7.32±0.53
5.	80	79.25±0.43	9.97±0.37
6.	160	88.04±0.59	12.28±0.35
7.	320	93.76±0.42	14.03±0.53
		IC 50 = 21.64 µg/ml	IC50= 320 µg/ml

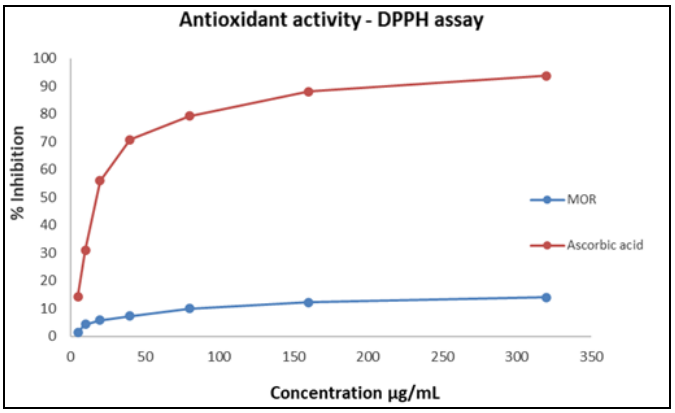

FIG. 2: ANTIOXIDANT ACTIVITY OF *MORINGA OLEIFERA* AQUEOUS LEAF EXTRACT AND ASCORBIC ACID

Physical Characteristics of Effervescent Granules of *Moringa oleifera*: Measurement of bulk density aims to determine the material bulk. This parameter described the volume that will be occupied by a certain number of granules, hence have an impact on determining dosage pack and the process of filling into the primary package. Furthermore, determination Hausner ratio were used to indirectly ascertain the flow characteristics therefore the interparticulate friction.

Flow characteristics can also be predicted through evaluation of the flowability and angle of repose. The evaluation results showed the three formulas included in the category could flow well (4-10 g/s). Also, the angle of repose showed all the granules could flow well (25-32°). Based on the results obtained, it was concluded that the granules of formula F3 showed very good flow characteristics. Good flow characteristics have an impact on the weight diversity and uniformity of dosage content.

TABLE 5: PHYSICAL CHARACTERISTICS OF EFFERVESCENT GRANULES OF *MORINGA OLEIFERA* LEAF EXTRACT

Physiochemical evaluation	F1	F2	F3	Specification
Organoleptic parameter	Dark green granules	Dark green granules	Dark green granules	
Bulk density	0.5775±0.013	0.5882±0.022	0.5911±0.122	-
Tapped density	0.6339±0.024	0.6214±0.157	0.6623±0.105	-
Angle of repose	31.97^0	28.57^0	26.5^0	$25\text{-}35^O$
Hausner's Ratio	1.09	1.05	1.1	1.00-1.18
Flow rate	6.5 g/s	6.6 s/g	6.4 s/g	4-10 g/s
Moisture content (%)	3.62±0.09	4.01±0.04	3.50±0.02	3-5%
Effervescent time	195sec	180sec	140sec	2-5min
pH value	5.62 ± 0.04	6.02± 0.04	6.69 ± 0.07	5 - 7

Another parameter that determined the granule characteristics was the moisture content. Effervescent granules easily absorb moisture from the air. In general, the requirement for the moisture content of granules was around 3-5%. Based on the evaluation results, the three formulations had a moisture content that fulfills the requirements.

Particle size evaluation showed the largest proportion was in the particle size of 379µm, while 30% were in the size range of 351µm.According to previous studies, granules with a narrow size distribution improved the flow characteristics. Based on the effervescence time, pH value, taste, flow characteristics the formulation F3 was found to be the best formula compared to others.

CONCLUSION: In the present study with *Moringa oleifera* Aqueous leaf extract we report the presence of phenolics and antioxidants. we also hypothesize that antioxidant nature of *Moringa oleifera* leaf extract may be due to phenolic and flavonoid content. The effervescent granules of *Moringa oleifera* leaf extract can play a highly

significant role in food and pharmaceutical industry because the natural antioxidants are less harmful than synthetic antioxidants

Moringa leaf extract effervescent granule formula developed in this study had good flow characteristics, effervescent time, and fulfills quality requirements. Furthermore, formulation F3 showed better flow characteristics than the other formulas. Also, results of the organoleptic and sensory evaluation showed the three formulas were acceptable. In addition, formulation F3 was the most acceptable in terms of colour, taste, aroma, and texture.

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