ORIGINAL ARTICLE

Design and Development of Gastroretentive Tablets of *Coccinia grandis* leaf extract for treating *Helicobacter pylori* infection

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Abstract

Aim: To design and develop a gastroretentive herbal formulation of *Coccinia grandis* for the treatment of *Helicobacter pylori* infection. **Material and Methods:** *C. grandis* extract was obtained by soxhlation process with ethanol as the menstrum. The herbal gastroretentive tablets containing 200 mg of *C. grandis* ethanolic extract were prepared by direct compression method using effervescent technique. HPMC K4M, HPMC K100M, Carbopol and guar gum were used as retarding polymers at various concentrations. Sodium bicarbonate was used at 20%w/w as gas generating agent and Avicel at 35%w/w as diluent. FTIR and DSC methods were employed to investigate the drug-excipient interactions. The formulated tablets were evaluated for various quality control parameters. **Results and Discussion:** The FTIR and DSC study revealed that there was no interaction between the extract and the excipients. All the formulated tablets were within the quality control limits. The kinetic studies specified that the release of extract from the gastroretentive tablets was by erosion-controlled diffusion mechanism. **Conclusion:** The formulation having the combination of gum with HPMC K15M exhibited minimum floating lag time and good release pattern which followed first-order kinetics.

Key words: *Coccinia grandis*, gastroretentive, *Helicobacter pylori*, herbal extract, resistance

INTRODUCTION

Warren and Marshall identified *Helicobacter pylori*, a Gram-negative bacterium, which is one of the most chronic pathogens in humans.[1] The World Health Organization categorized *H. pylori* as Class 1 carcinogen.[2] About 50% of the world population are infected with *H. pylori* and occurrence is more in developing countries than in developed countries.[3] *H. pylori* infection persists throughout life as an asymptomatic condition. However, their presence in the stomach can cause chronic gastric inflammation and leads to severe gastric diseases such as peptic ulcer to tissue lymphoma. The abolition offers the most direct approach to reduce the gastric and peptic ulcer prevalence in high-risk population. The humans may have more than one strain of *H. pylori* and many strains are resistant to commonly used antibiotics. The international guidelines for treating the patients diagnosed with *H. pylori* infection are by triple therapy. It consists of proton pump inhibitor (PPI), clarithromycin, and amoxicillin for 7–14 days. The cure rate observed was less due to *H. pylori* resistance to clarithromycin. Hence, second-line therapy was proposed, comprising a PPI with two or three antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline. The *H. pylori* organism exhibiting biofilm formation by that showing the drug resistance.[4,5] Hence, there is a vast need to find out new and efficient treatment techniques against *H. pylori* infection. Plants exhibit a wide range of pharmaceutical action similar to modern medicine. This is due to their secondary metabolites; they have the ability to overcome the resisting antibiotic monotherapy. Biofilms are communities of microbes attached on the surface. *H. pylori* form biofilms on the surface of gastric mucosa and showing resistance.[6] Hence, antibiofilm formers are required for eradication of *H. pylori*. The residence of *H. pylori* is in the stomach region. Hence, the formulations which showing more residence time in the stomach are suitable to eradicate the *H. pylori*.[7]

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Coccinia grandis (L) Voigt (*Cucurbitaceae*) is a perennial climber commonly known as Ivy gourd and Scarlet gourd, primarily grown in tropical climates and can be found cultivated as a vegetable crop.[8,9] This plant is the native of Africa and Asia, including India, the Philippines, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam, and Eastern Papua New Guinea and also found in the Northern Territories of Australia has its own importance in the traditional medicine in various places. Every part of this particular plant has its own pharmacological importance. Moreover, these plant fruits were considered a vegetable has rich nutritional values. This plant showing good antibiofilm capacity.[10,11] Hence, in the present research work, gastroretentive formation of the ethanolic extract of *C. grandis* was designed for the eradication of *H. pylori* infection.

MATERIALS AND METHODS

Materials

Leaves of the plant *C. grandis* are collected directly from the plant in and around Guntur, Andhra Pradesh, India, from June to October. The shade dried leaves were grounded into a coarse powder. Dr. Ammani, Head, Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, confirmed the authentication of the plant. The ethanolic extract of the plant was prepared in the laboratory. Amoxicillin obtained from Dr. Reddy's Laboratories, hydroxypropyl methylcellulose (HPMC) K_4M, HPMC $K_{100}M$, Carbopol, and guar gum were purchased from Merck Labs, Mumbai, Sodium bicarbonate, citric acid from Fisher Scientific, Mumbai. All the ingredients are of pure and of analytical grade.

Methods

Extraction

The extraction is carried out in a Soxhlet extractor with ethanol as the menstruum. The extract was concentrated by the distillation process. Then, the extract was dried under the shade for further evaporation of the solvent. The dried extract was labeled and stored in desiccators for further use. The extract was tested for various phytochemical constituents and the data are given in Table 1.

Extract-excipient compatibility study

Extract and excipient compatibility study was performed for the physical mixture of an ethanolic extract with various polymers in the ratio 1:1. The physical mixture samples were subjected to Fourier-transform infrared (IR) spectral studies using KBr pellet method. Spectra of drug and polymer were taken and analyzed for the major interactions.

Micromeritic properties

The pure ethanolic extract and blend of the formulations were evaluated for various micromeritic properties such as angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio. The bulk and tapped density of the powder was determined using 25 ml measuring cylinder. The volume occupied by the blend was measured and bulk density was reported in g/ml. The blend containing cylinder was tapped until the constant volume was obtained and the true density was calculated in g/ml. The angle of repose was calculated by hopper method. The height (h) and radius (r) of the heap were noted and the angle of repose was calculated using the formulae $\tan \theta = h/r$. The percentage of compressibility was calculated from the difference between the true and bulk density divided by the true density.[12,13] The micromeritic properties of the blend data are given in Table 2.

Preparation of gastroretentive tablets

The herbal gastroretentive tablets containing 200 mg of *C. grandis* ethanolic extract were prepared by direct compression method by effervescent technique.[14] HPMC K_4M, HPMC $K_{100}M$, Carbopol, and guar gum were used as retarding polymers at various concentrations. Sodium bicarbonate was used at 20%w/w as gas generating agent, Avicel at 35%w/w as diluent, 1%w/w of talc as glidant, and 1%w/w of magnesium stearate as lubricant. All the ingredients were accurately weighed and mixed geometrically after passing the ingredients through sieve No. 44. The blend was compressed into tablets using a rotary compression machine having 12 mm tooling set. The formulae are given in Table 3.

Table 1: Preliminary screening of ***Coccinia grandis*** leaves extract

Bioactive constituents	Ethanol	Methanol	Chloroform	Aqueous
Alkaloids	+	+	+	_
Flavonoids	+	+	_	+
Carbohydrates	_	+	+	+
Glycosides	+	+	_	+
Saponins	+	+	_	+
Steroids	+	-	-	-
Tannins	+	-	-	-
Triterpenoids	-	+	+	-

Table 2: Micromeritic properties data of the powder blend

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility Index (CI) (%)	Hausner's ratio (HR)	Angle of repose (θ)
Herbal extract	0.3503	0.4671	8.77	1.09	28
F1	0.304	0.348	12.6	1.144	27.8
F2	0.306	0.348	12.06	1.137	28.6
F3	0.308	0.350	12	1.136	29.4
F4	0.311	0.356	12.64	1.144	27.12
F5	0.315	0.362	12.98	1.149	29.64
F6	0.312	0.354	11.86	1.134	30.45
F7	0.314	0.358	12.29	1.140	29.89
F8	0.315	0.362	12.98	1.149	30.25

Table 3: Formulae of gastroretentive herbal tablets

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)
Herbal Extract	200	200	200	200	200	200	200	200
HPMC K_4M	40	-	-	-	-	-	-	-
HPMC $K_{100}M$	-	40	40	40	-	-	40	40
Carbopol 934	-	-	40	-	-	-	-	-
Carbopol 940	-	-	-	40	-	-	-	-
Guar gum	-	-	-	-	40	-	40	-
Xanthan gum	-	-	-	-	-	40	-	40
Ethyl Cellulose	120	120	120	120	120	120	120	120
Avicel	85	85	85	85	85	85	85	85
Sodium bicarbonate	40	40	40	40	40	40	40	40
Citric acid	20	20	20	20	20	20	20	20
Magnesium stearate	3	3	3	3	3	3	3	3
Talc	6	6	6	6	6	6	6	6
Total	514	514	554	554	514	514	554	554

HPMC: Hydroxypropyl methylcellulose

Evaluation of Tablets[15,16]

The formulated tablets were evaluated for various quality control parameters such as weight variation, content uniformity, hardness, friability, disintegration, and for *in vitro* drug release parameters.

Weight uniformity

Twenty tablets were selected randomly and weighed individually, the average weight was calculated, and the individual weights were compared with the average weight.

Hardness test

The hardness or crushing strength of the tablets were determined individually by Pfizer hardness tester. The mean hardness was calculated and reported in kg/cm^2.

Friability

The friability of the tablets was determined by using Roche Friabilator. Weighed tablets (W0) were transferred into friabilator and were operated at 25 rpm for 4 min or up to 100 revolutions. The intact tablets were weighed again (W). The percentage friability was calculated using the formulae:

$$F = \frac{W0 - W}{W0} \times 100$$

Drug content

This test was performed by selecting 10 tablets randomly from the batch. The amount of *C. grandis* ethanolic extract present in each tablet was estimated by dissolving it in 0.1N HCl. The solution was filtered, diluted, and estimated analytically using an ultraviolet-visible spectrophotometer at 271 nm.

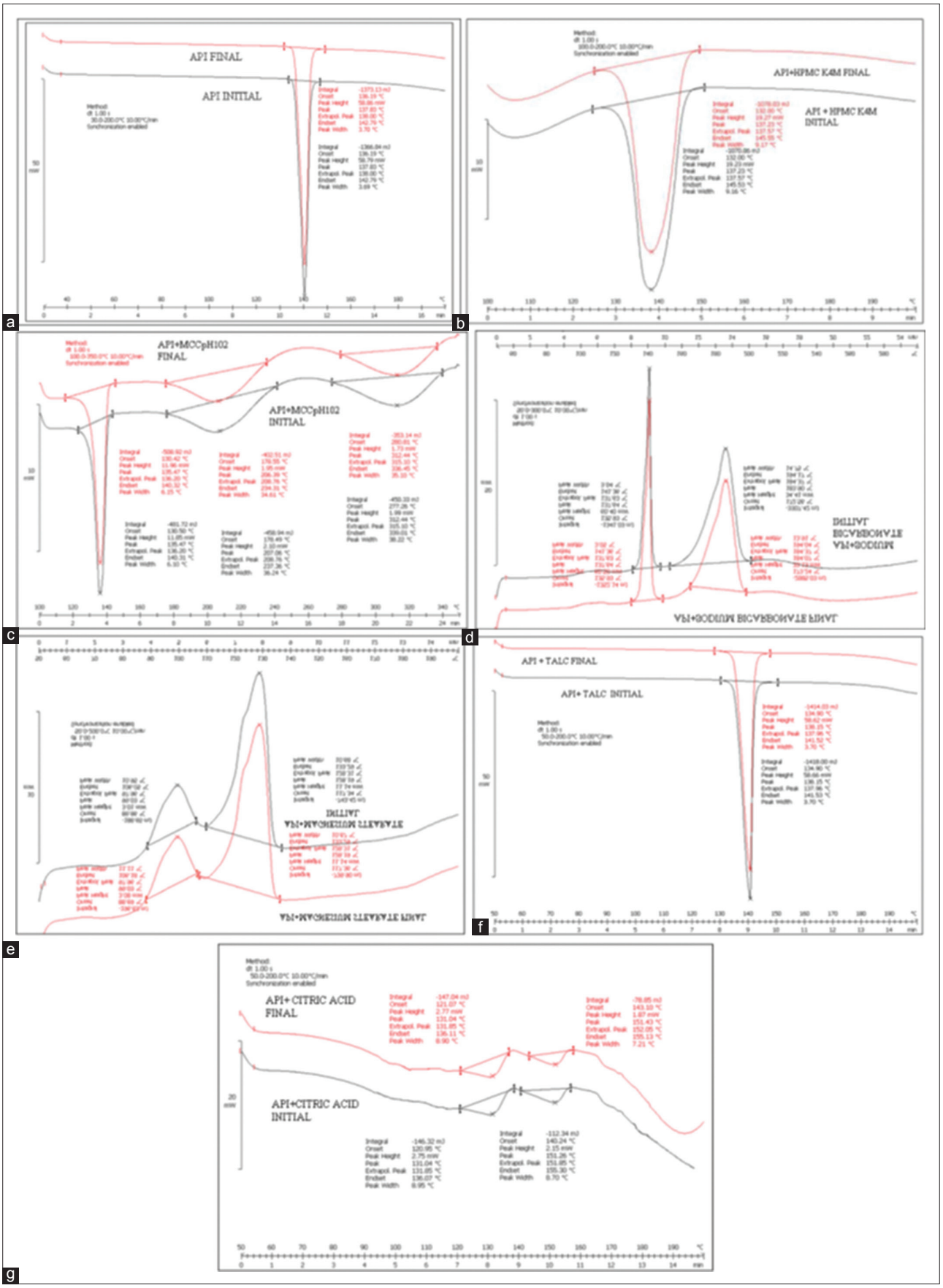

Figure 1: Differential scanning calorimetry thermogram of (a) pure extract (b) extract + hydroxypropyl methylcellulose (c) extract +microcrystalline cellulose (d) extract+ sodium bicarbonate (e) extract + magnesium stearate(f) extract +Talc (g) extract + citric acid

Swelling index study

The swelling index of the formulated tablets was measured by studying its weight gain. It is measured by placing the tablets in a Petri dish containing 0.1N HCl. The tablet weight measured after 1, 2, 3, 4, and 5h withdrawing tablet and blotted with tissue paper to remove the excess fluid and weighed using analytical balance.[17] The swelling index was calculated using the formulae;

$$\text{Swelling index} = \frac{\text{wet weight of tablet} - \text{dry weight of tablet}}{\text{dry weight of tablet}} \times 100$$

In vitro floating studies

The *in vitro* buoyancy was determined by floating lag time method as per the method described by Rosa *et al*. The tablets were placed in a 100 ml beaker containing 0.1N HCl. The time required for the tablets to rise to the surface to float was determined as floating lag time, and the period the tablet constantly present on the surface of the dissolution medium was noted as floating time.[18,19]

In vitro drug release studies

The drug release studies from different formulated tablets were performed using IP type I dissolution rate testing apparatus in 900 ml of 0.1N HCl as dissolution medium with an rpm of 50, maintained at 37±0.5°C. Samples were collected at regular intervals of time and replaced with an equivalent volume of fresh dissolution medium. The samples were analyzed at a wavelength of 271 nm using ultraviolet spectrophotometer. The process was carried out in triplicate.

The dissolution data of the formulations were fitted to various release kinetics models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas equation models. The order of drug release from the matrix formulations was described using zero or first order kinetics. The mechanism of drug release was explained by Higuchi or Korsmeyer–Peppas equation.

RESULTS AND DISCUSSION

The present research work was aimed to formulate herbal gastroretentive dosage form of *C. grandis* extract for treating *H. pylori* infection. The literature revealed that *C. grandis* extract having a good effect on *H. pylori* and preventing the biofilm formation of *H. pylori*. The *H. pylori* organisms are residing in the stomach. Hence, in the present research floating tablets were designed with the effervescent approach, because they are easy to fabricate and produce buoyancy in the gastrointestinal tract. The floating tablets were formulated by direct compression method using three polymers such as HPMC, guar gum, and Carbopol at various concentrations.

The dried leaves of *C. grandis* were extracted by soxhlation process using ethanol as the menstruum. The extract was dried and tested for the phytochemical constituents. The results of the preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, and steroids. The results were shown in Table 1. The compatibility of an ethanolic extract with the polymers was studied using IR spectroscopic method and differential scanning calorimeter (DSC) thermogram method. The DSC thermograms are shown in Figure 1. IR spectra of the formulations are given in Figure 2. The spectra revealed that optimized formulations are having characteristic peaks the same as that of the pure extract. It indicated that there is no interaction between drug and excipients. The results indicated that the melting point of the extract is at 140°C. The thermograms of the solid mixtures of various excipients

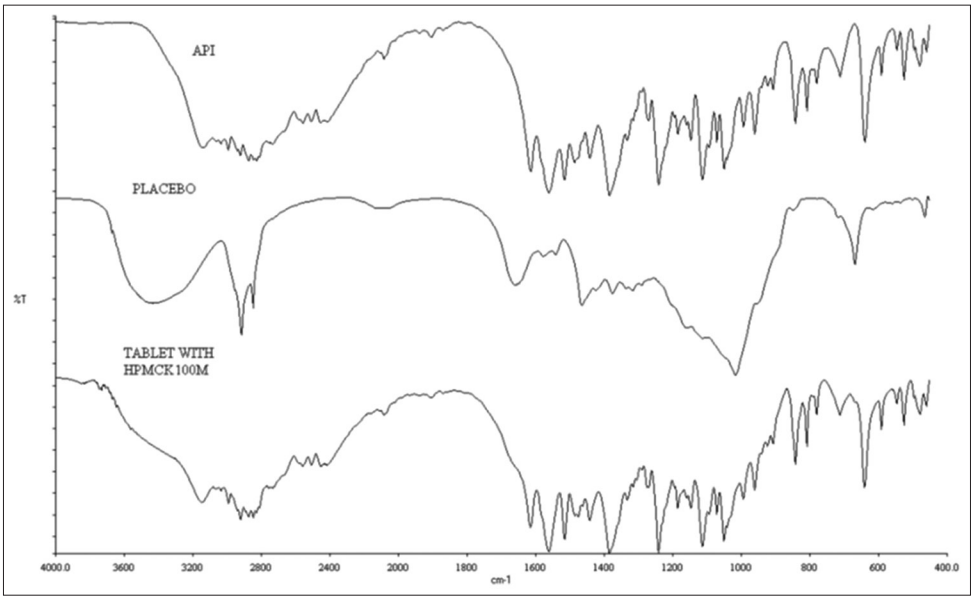

Figure 2: Infrared spectra of pure extract and herbal tablet

analyzed after storage of 3 months. All the blends showed a peak near to 140°C with almost same normalized energy, which indicated that the extract structure is not affected by the presence of various excipients used in the preparation of floating tablets. The results indicated that there was no interaction between the extract with a polymer.

Micromeritic properties of the blend were performed by measuring various parameters such as angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio. The data are given in Table 2. The bulk density and the tapped density for all formulations were found to be within limits. Carr's index and Hausner's ratio were found to be in the range of 11–12 and 1.13–1.14, respectively, indicating good flow. The angle of repose is in the range of 27–30. The data revealed that the blend exhibited good compressibility and flow nature. Hence, the tablets were formulated by direct compression method.

All the formulated tablets were evaluated for various quality control parameters as specified in pharmacopeia. The quality control tests data of the tablets are given in Table 3. All the batches of tablets were found to be in the range of 4–6 Kg/cm^2, friability of the all the formulations was found to be < 1% and the drug content was in the range of 97–103.5%. It indicated that all the herbal floating tablets formulated by employing different concentrations of polymers were of good quality and all are within the official specifications regarded to weight variation, friability, hardness, and drug content.[20,21] The data of the quality control parameters are given in Table 4.

The formulations were subjected to *in vitro* buoyancy studies, and the results are given in Table 5. Sodium bicarbonate (20%) was used as a gas generating agent. The floating tablets which are formulated with HPMC K4M are shown good lag time with floating time of 6 h after dispersion. The F2 formulation showed lag time of 35 s and was dispersed after 6 h the formulation containing HPMC with synthetic polymer, i.e., Carbopol shown good lag but shown floating time of 8 h. Hence, in the next formulations combination of gums with HPMC were tried. These formulations (F7, F8) shown good lag time and floating time, i.e., more than 12 h. The floating lag time for all the formulations was <2 min. The *in vitro* floating buoyancy data and the percentage swelling index data of all the formulations are given in Table 5. All the formulations were stable during the study, due to a sufficient amount of the polymer to maintain the matrix integrity. The swelling behavior of all the formulations was due to the formation of the hydrogel by hydrophilic polymer, and the type and concentration of polymer increases, the percentage swelling index of the tablets was found to increased.[22,23] The swelling data of the formulations are given in Table 5.

All the formulations were subjected to *in vitro* dissolution studies in 0.1 N HCl, i.e., pH 1.2 buffer. The present investigation revealed that natural, semisynthetic, and synthetic polymers are suitable for the formulation of gastroretentive herbal tablets. The study data revealed that the formulations F1, F2 shown the ability to retain for 8 h, F3-F5 for 10 h, and the formulations F6-F8 shown the release more

Table 4: Physical characteristics of gastroretentive herbal tablets

Batch No	Weight variation (mg)	Thickness (mm)±SD	Density (g/cc)	Hardness (kp)±SD	Friability (%)	Drug content (%)±SD
F1	511.9±1.744	3.71±0.02	0.897	18.44±0.30	0.52	99.89±0.73
F2	510.8±1.899	3.69±0.03	0.897	18.12±0.27	0.61	100.56±0.78
F3	552.45±1.830	5.82±0.01	0.880	18.05±0.31	0.54	100.88±0.54
F4	554.25±1.77	5.81±0.05	0.897	18.25±0.24	0.59	99.98±0.28
F5	512.5±1.568	3.85±0.02	0.872	18.55±0.18	0.68	100.21±0.26
F6	511.9±1.885	3.78±0.01	0.895	18.38±0.25	0.58	99.67±0.42
F7	551.54±1.638	5.87±0.11	0.884	17.38±0.12	0.59	100.32±0.51
F8	554.78±1.830	5.82±0.02	0.888	19.25±0.25	0.62	100.65±0.12

Table 5: *In vitro* floating buoyancy data of gastroretentive herbal tablets

Batch Number	Floating lag time (s)±SD	Floating time (h)	Matrix Integrity	% Swelling index
F1	20±0.11	8	+	78.35±0.25
F2	35±0.21	8	+	81.24±0.32
F3	50±0.41	8	+	84.23±0.74
F4	20±0.51	8	+	82.31±0.17
F5	40±0.21	8	+	79.85±0.34
F6	80±0.61	12	+	82.37±0.41
F7	20±0.71	12	+	83.49±0.74
F8	30±0.81	12	+	79.56±0.19

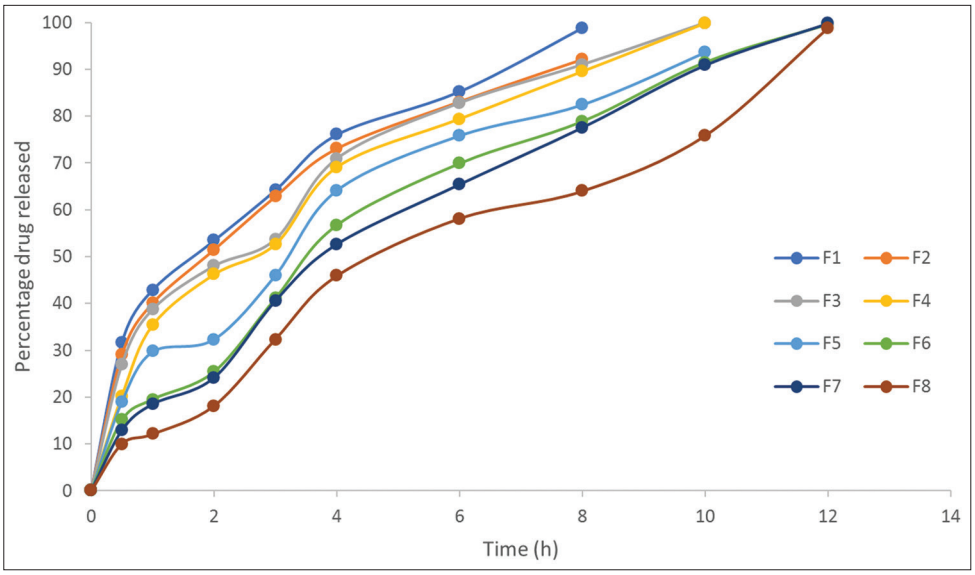

Figure 3: *In vitro* drug release profile data gastroretentive herbal tablets

than 12 h the dissolution profile is depicted in Figure 3. The release profile of herbal extract from all the formulations was best expressed by first-order release. The plots of Higuchi were linear, indicating that release of the extract from herbal formulations was by diffusion mechanism. Based on Korsmeyer–Peppas equation "*n*" values were within 0.541–0.656 indicating the non-Fickian diffusion, which means diffusion and erosion-controlled rate of release.

CONCLUSION

The present work was aimed to design gastroretentive formulation for the ethanolic extract of *C. grandis* for the treatment of *H. pylori* infection in an effective manner by preventing the biofilm formation. The gastroretentive formulations were designed using natural, semisynthetic, and synthetic polymers alone or in combination. All the formulation having 20% sodium bicarbonate as gas releasing agent, 30% microcrystalline cellulose as diluent and ethyl cellulose as a retarding agent. The formulation having the combination of gum with HPMC K15M exhibited the good floating lag time and shown good release pattern following first-order kinetics. The DSC and IR studies revealed that there was no interaction between the drug and excipients in the formulation.

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