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Formulation and Evaluation of Sichuan Pepper Extract-loaded Chewable Jelly For Anti-inflammatory Activity

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Abstract

Background: The interest in herbal formulations as safer alternatives for treating inflammation is increasing. Natural bioactive compounds from Sichuan pepper are reported to have anti-inflammatory properties. To improve patient adherence and compliance, development of a palatable dosage form like chewable jelly can be done. **Aim:** This study aimed to prepare a chewable jelly with Sichuan pepper extract and then evaluate its physical and chemical properties and anti-inflammatory effects. **Methods:** Sichuan pepper was extracted with ethanol by maceration for 72 h. Chewable jelly formulations were prepared by the heating-congealing method using gelatin, citric acid, propylene glycol, sucrose and sodium benzoate. Different batches were prepared and evaluated for physicochemical properties like pH, viscosity and appearance. The drug release was studied in phosphate buffer (pH 6.8). The anti-inflammatory activity was determined by the protein denaturation inhibition method using Diclofenac as a reference standard. Stability studies were carried out at two temperatures for 4 weeks, namely 25°C and 40°C. **Results:** The physicochemical characteristics of the formulations F1 and F2 were satisfactory, with adequate pH, good consistency and uniform appearance. These formulations exhibited comparatively better drug release profiles in phosphate buffer pH 6.8. The anti-inflammatory study showed that the extract exhibited moderate activity with an IC_{50} value of 450 ppm, whereas diclofenac showed better activity with an IC_{50} of 281 ppm. The optimised formulation was stable for the entire period of the study with no significant changes.

Major Findings: A novel patient-acceptable dosage form, i.e., chewable jelly, was successfully formulated as a potential herbal alternative showing promising anti-inflammatory activity. Further *in vivo* and clinical studies are required to confirm its efficacy and safety.

Keywords: Anti-inflammatory, Chewable Jelly, Herbal Extract, Medicated Jelly, Sichuan Pepper

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1. Introduction

Sichuan pepper belongs to the *Zanthoxylum* genus, which represents the Rutaceae family and has more than 250 species[1]. It holds considerable significance for culinary and medicinal purposes in China and is cultivated and eaten in many Asian nations[2]. Research has demonstrated that extract from Sichuan pepper is a natural anti-inflammatory agent. Its rich combination of bioactive phytochemicals and ability to change bioactive phytochemical pathways are both strongly linked to reducing inflammation. Studies on Sichuan pepper have demonstrated that its extract mitigates inflammation by regulating and persistently inhibiting TLR-4-dependent cytokine release and significant inflammation suppression pathways, including PPARγ and NF-κB. Compounds such as limonene, methyl cinnamate, tambulin, and bergapten from Sichuan pepper extract, alongside the extensively researched spice compound linalool, have exhibited direct

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anti-inflammatory effects accompanied by a significant decrease in oxidative stress in standard studies. The evidence supports the utilisation of a multi-targeted strategy as a foundation for the formulation of a plant-derived anti-inflammatory agent. In addition to its medicinal uses, Sichuan pepper is a spice and condiment in almost all Asian countries. In China, it is one of the "eight famous condiments." The dried husks of *Zanthoxylum bungeanum* Maxim. People also like *Zanthoxylum schinifolium, Sieb.* et Zucc. and other spices that are similar because they have strange pungent and tingling properties[3]. Chemoselective ion channels TRPA1 and TRPV1 are the sensory properties that result from the hydroxy-sanshool compounds. The structure of the compounds, and especially the presence of specific double bonds and amine groups, is critical for the creation of tingling, salivation, and numbness[4]. Sichuan pepper's therapeutic value is attributed to the wide range of bioactive compounds. Among the bioactive compounds, there are 140 that are known for their biological activity related to anti-inflammatory, analgesic, antibacterial, antioxidant, and anticancer effects[5]. The bioactive compounds enhance the spice's medicinal properties and validate the traditional use of the spice in various health conditions[6]. Similarly, species like *Zanthoxylum armatum* also have a similar effect.

1.1 Jelly

Jellies are semisolid medications, which could be either clear, translucent, or non-oily, and are used both internally and externally. Due to their smoothness and pleasant texture, they are ideal for easy consumption by patients[7]. Various types of dosage forms are available, but the jelly form is believed to be highly suitable for patients who suffer from dysphagia, since it is a sticky fluid or semi-solid substance that can be swallowed or chewed with ease[8]. Administration through the oral route has been identified as the best choice among other routes of drug administration due to its easy applicability, non-invasiveness, and improved patient compliance. From this perspective, medicated oral jellies have become increasingly popular, since they do not require the use of water during administration[9]. Medicated jellies possess many benefits compared to traditional dosage forms. Since they are soft and smooth in nature, they can easily be chewed by aged and paediatric patients. Children often like jelly preparations better than tablet dosage forms due to their appealing appearance and tasteful flavour. Furthermore, they play an important role in treating dry mouth conditions by inducing salivation and also serve the purpose of treating systemic conditions as well as oral disorders[10].

However, conventional oral dosage forms like tablets and capsules may pose problems like dysphagia, poor palatability, and delayed effects[11]. Dysphagia is a very common problem, occurring in almost all ages, with around 35% incidence among the general population, 60% of institutionalised elderly, and 18-22% cases among people residing in nursing homes[12]. In terms of the market share of medicated jellies in the world market, the United States tops the list with around 20% market share. In order to address the above issues, technologists in the pharmaceutical field have invented a new oral dosage form called 'Oral Medicated Jellies (OMJs)'. Such formulations will disintegrate very fast in saliva, without requiring any additional fluid and can dissolve and get absorbed rapidly in the blood, leading to an increased availability of the drug and quick action[13]. In addition to medical applications, jelly delivery systems have also found extensive applications in the confectionery field. Jelly confections have become a popular snack among all age groups and are formulated with the aid of gel-forming agents like gelatin, starch, pectin, sucrose, glucose syrups, acids, colouring agents and flavouring agent[14]. Due to these advantages, orally medicated jellies have come to be regarded as a potentially attractive dosage form for a variety of patient categories, particularly those involving children and dysphagia patients. The importance of these preparations can be further highlighted by the fact that they find mention in the Japanese Pharmacopoeia (16th Edition – implemented since 2011)[15]. Jellies are classified as Medicated Jelly, Lubricating Jelly and Miscellaneous Jelly[16,17].

2. Materials and Methods

2.1 Materials

Sichuan pepper seeds were purchased from Munsyari, Uttarakhand, India. The gelatin, citric acid, methyl paraben, and sodium benzoate were issued from the central store of the college (SGRRU) and were of

analytical grade. All chemicals and reagents were of analytical grade and were procured from authorised dealers.

2.2 Preparation of Seeds Powder

The dried seeds were finely ground in a mechanical grinder. Sieving was carried out using a No. 40 sieve. The resulting powder was kept in an airtight bottle for subsequent analysis[18].

2.3 Preparation of Extract

The coarsely powdered seeds (50 g) were extracted with 70% ethanol (v/v) by the cold maceration process. The powder was soaked in the solvent in a 1:10 w/v ratio with occasional stirring in a beaker for 72 h. After completion of the extraction, it was filtered through muslin cloth or Whatman filter paper and the solvent was removed by evaporation to dryness on a water bath, and it was stored in a desiccator[18].

2.4 Method of Preparation of Chewable Jelly[8-10,17]

The process of making jelly involves heating and congealing processes. The procedure for making jelly is as follows:

All the ingredients required for the formulation were accurately weighed as shown in Table 1. Sucrose syrup was prepared by dissolving sucrose in water and heating the mixture to 60–70 °C until the sucrose was completely dissolved. Citric acid was then added to prevent sugar crystallization. Gelatin was hydrated by soaking it in the required quantity of water and was subsequently added to the syrup with continuous heating until it dissolved completely. Sodium benzoate, previously dissolved in a suitable vehicle, was incorporated into the mixture with constant stirring. The required amount of Sichuan pepper extract was then added to the warm mixture and stirred thoroughly to ensure uniform dispersion throughout the formulation. Sodium citrate was added with continuous stirring to maintain the desired pH. Finally, the color and flavoring agents were incorporated and mixed thoroughly. The resulting mixture was poured into silicone molds and allowed to cool at room temperature until completely solidified. The jellies were then removed from the molds, wrapped in gelatin paper, and stored in a dry place.

2.5 Calibration Curve of Sichuan Pepper Extract[19]

2.5.1 Preparation of Stock Solution

The extract was weighed carefully, amounting to about 100 mg, then diluted in ethanol in a 100 ml volumetric flask. This produced a solution with a concentration of 1000 µg/ml, which was referred to as the stock solution.

2.5.2 Preparation of Working Standard Solution

The working standard solution was obtained by taking 10 ml from the stock solution and diluting it to 100 ml using 6.8 pH phosphate buffer in a volumetric flask. The resulting solution had a concentration of 100 µg/ml.

Table 1. The components of different formulations of chewable jelly

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Extract (g)	0.5	1.0	1.5	2.0	2.5	0.5	1.0	1.5	2.0	2.5
Gelatin (g)	1	1.5	2	2.5	3	2	4	6	8	10
Sucrose (g)	35	35	35	35	35	35	35	35	35	35
Citric acid (g)	0.5	0.5	0.5	0.5	0.5	0.7	0.7	0.7	0.7	0.7
Propylene glycol (g)	5	5	5	5	5	5	5	5	5	5
Sodium benzoate (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium citrate (g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Flavouring agent (g)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Colouring agent (g)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water (q.s to 100 ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.5.3 Preparation of Serial Dilution

Approximately 0.5, 1.0, 1.5, 2.0, and 2.5 ml from the standard solution were taken using the pipette, and their volume was adjusted to the 10 ml level using buffer at pH 6.8 so as to get solutions in the concentrations of 2-16 µg/ml.

3. Evaluation Parameters of Chewable Jelly

3.1 Organoleptic Properties[19]

Appearance: To evaluate the colour, clarity and uniformity of the jelly.

Texture: To evaluate the smoothness, firmness and consistency.

Odour: To evaluate the pleasantness and absence of rancidity.

Taste: To evaluate the sweetness, bitterness, or any off-flavours.

3.2 pH

The measurement of the pH value of jelly can be performed using a digital pH meter. For this, mix 50 ml distilled water with 0.5 g of jelly. Measure the pH[20,21].

3.3 Viscosity

It was measured using a Brookfield viscometer. Brookfield viscometer at 50 rpm at 25°C was used for the viscosity test[20,21].

3.4 Weight Variation

Approximately twenty samples of medicated jelly were selected, and their weight was measured separately using an analytical balance. Average weight and % variation in weight were calculated.

3.5 Thickness

Using a Vernier caliper measure the thickness. It should be expressed in mm[22].

3.6 Grittiness and Stickiness

Stickiness and grittiness of jelly can be identified by visual examination. This is done by rubbing the jelly samples on each other between the thumb and index finger.

3.7 Syneresis

Syneresis occurs when the jelly gels contract and separate water. This phenomenon will occur especially if a small amount of gelling agent was used. All types of jelly samples were checked at room temperature (25°C and 5°C) and 8°C, 1°C for syneresis[20].

3.8 Spreadability

The spreadability test involved the placement of jelly between two glass plates that were then pressed against each other with a 1000 gm load for 5 mins. The indicator of spreadability is the time taken to separate the two glass plates[20].

Spreadability is calculated as:

$$S = W \times L/T$$

Where: S – Spreadability; W - Weight tied to upper slide; L - Length of a glass slide; T - Time required to separate 2 slides.

3.9 Stability Study

The stability study of the formulations was done on the four formulations (F1, F2, F3, and F4). Jellies were carefully packed and kept under both conditions (25°C and 40°C) for four weeks. Changes in the physical parameters (appearance, stickiness and grittiness, pH, viscosity, thickness, weight variation, and drug concentration) were noted at the 4th week[22].

3.10 Disintegration Test

Disintegration test is done using the disintegration apparatus of USP to measure the disintegration time of jellies. Disintegration time of jellies is recorded in pH 6.8 phosphate buffer or artificial saliva at 37°C[23].

3.11 *In Vitro* Dissolution Study

The dissolution study of the formulated medicated jellies was done using two different methods. In the case of dissolution of the medicated jelly, the USP type II dissolution apparatus (paddle) was used as a method of dissolution. 900 ml of 6.8 pH buffer solution was taken as the dissolution medium and maintained at 37 ± 0.5°C with a paddle speed of 50 rpm. 5 ml of the sample solution was withdrawn from the medium at 5, 10, 20, 30, and 45 min intervals. The same amount

of buffer solution was added to maintain the volume. Filtration, dilution, and spectrophotometric analysis at 270 nm were done[10].

3.12 Anti-inflammatory Activity Test using Protein Denaturation Method

Protein source was provided in the form of fresh egg albumin solution (1-2% v/v). The reaction mixture was prepared in such a way that out of a total of 5 ml, 0.2 ml was egg albumin, 2 ml was jelly extract of different concentrations, and 2.8 ml was phosphate-buffered saline (PBS; pH 7.4). The standard used was diclofenac sodium. The control solution was made from a combination of 0.2 mL egg albumin, 2.8 ml PBS (pH 7.4), and 2 ml triple-distilled water. All solutions were incubated at 37±2°C for 30 min and then heated to 70±2°C for 15 min.

The percentage inhibition of protein denaturation was calculated using:

$$\%\text{Inhibition} = \frac{(A_c - A_S)}{A_c} \times 100$$

where: A_c = Absorbance of control; A_s = Absorbance of sample; IC_{50} values were determined from the plot of % inhibition *vs.* concentration.

4. Results and Discussions

4.1 Preformulation Studies

4.1.1 Organoleptic Properties

In this study, chewable jelly was made from Sichuan pepper powder with the use of gelatin as the gelling agent. The organoleptic characteristics include the square shape, yellow transparency, a mild to moderately strong aroma, and sweetness. Homogeneous appearance is considered beneficial for consumers' perception and acceptance of the product. It was also soft, smooth, and not sticky. Tables 2-4 present the results of the evaluation of the organoleptic characteristics, preliminary phytochemical screening of the extract and physical appearance of the prepared chewable jelly, as shown in Figure 1.

4.1.2 Solubility Test

As for the solubility test, it was found that the extract of Sichuan pepper powder was poorly soluble in water, since there was only minor dissolution and slight haziness when tested in distilled water and in phosphate buffer (pH 6.8). However, greater solubility was seen in organic solvents like methanol, ethanol, and acetone, where clear solutions were obtained, as shown in Table 5. Clearly, the extract was poorly soluble in water but more soluble in organic solvents.

Table 2. Results of organoleptic properties of jelly

Formulation	Colour	Odour	Texture	Taste	Shape
F1	Yellow	Mild	Soft	Sweet	Square
F2	Light yellow	Mild	Soft	Sweet	Square
F3	Light yellow	Mild	Good	Sweet	Square
F4	Yellow	Moderate	Firm	Sweet	Square
F5	Yellow	Moderate	Smooth	Sweet	Square
F6	Yellow	Mild	Smooth	Sweet	Square

Table 3. The result parameter of the physical appearance of chewable jelly

Formulation	Transparency	Consistency	Stickiness	Grittiness	Syneresis
F1	Translucent	Smooth	Non sticky	No grittiness	No syneresis
F2	Translucent	Too soft	Non sticky	No grittiness	No syneresis
F3	Translucent	Smooth	Non sticky	No grittiness	No syneresis
F4	Translucent	Smooth	Non sticky	No grittiness	No syneresis
F5	Translucent	Smooth	Non sticky	No grittiness	No syneresis
F6	Translucent	Smooth	Non sticky	No grittiness	No syneresis

4.1.4 Standard Curve of Sichuan Pepper Extract

A calibration curve of Sichuan pepper extract was plotted for concentrations between 2 to 16 µg/ml using phosphate buffer, distilled water, and ethanol solutions, and the λmax of Sichuan pepper extract was found to be 270 nm (Figure 2). In distilled water, a linear relationship was obtained between absorbance and concentration. The obtained regression equation was $y = 0.0565x + 0.00242$, while the R^2 value was 0.9959, as shown in Figure 3 showing good linearity. But the absorbance values were relatively low, as the compound shows poor solubility in aqueous solution. On the other hand, in ethanol, the calibration curve showed high absorbance values at each concentration level. The regression equation for the obtained results was $y = 0.056x + 0.0289$ with an R^2 value of 0.9963, as shown in Figure 4, showing very good linearity.

Table 4. Preliminary phytochemical screening of Sichuan pepper extract

S. No.	Phytoconstituent	Tests	Result
1	Carbohydrates	Molisch's test	+
2	Proteins	Biuret test	-
3	Steroids	Liebermann-Burchard	+
4	Glycosides	Keller-Killiani test	+
5	Saponins	Foam test	+
6	Alkaloids	Dragendroff's test	+
7	Tannins	Ferric chloride test	+
8	Phenolics	Lead acetate test	+
9	Flavonoids	Shinoda test	+
10	Terpenoids	Salkowski test	+

4.2 Evaluation Parameters of Jelly

4.2.1 Hardness, Chewiness, Gumminess

The hardness of the gel formulations gradually increased from F1 to F5, implying that the increase in the amount of polymer resulted in firming up the structure. Again, an increase was noticed but not necessarily in a linear range. Relatively lower standard deviation values imply good consistency within the

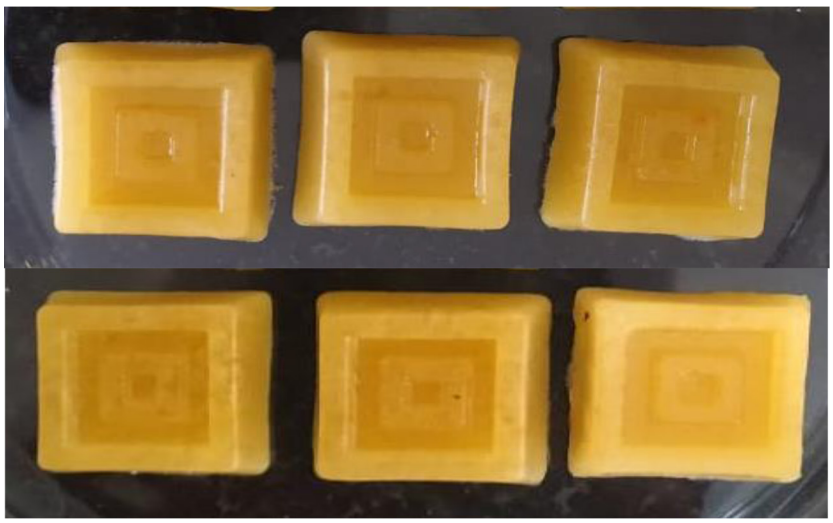


Figure 1. The physical appearance of gelatin-based Sichuan pepper extract chewable jelly.

Table 5. Solubility study of Sichuan pepper extract in different solvents

S. No.	Solvent used	Observation	Result
1	Distilled water	Slight turbidity observed	Slightly soluble
2	Ethanol	Clear solution formed	Sparingly soluble
3	Methanol	Transparent solution obtained	Sparingly soluble
4	Phosphate buffer pH (6.8)	Slight haze observed	Slightly soluble
5	Acetone	Soluble with clear appearance	Sparingly soluble

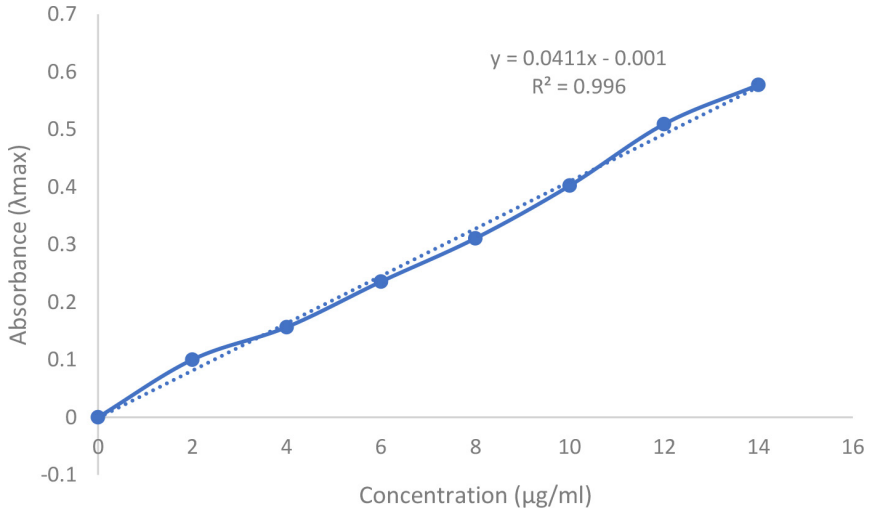


Figure 2. Standard curve of Sichuan pepper extract at 272 nm in pH 6.8.

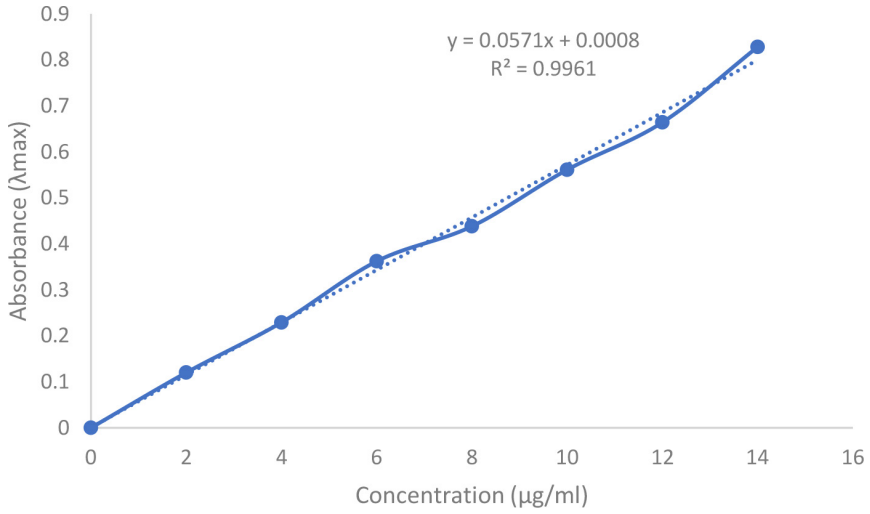


Figure 3. Standard curve of Sichuan pepper extract in distilled water.

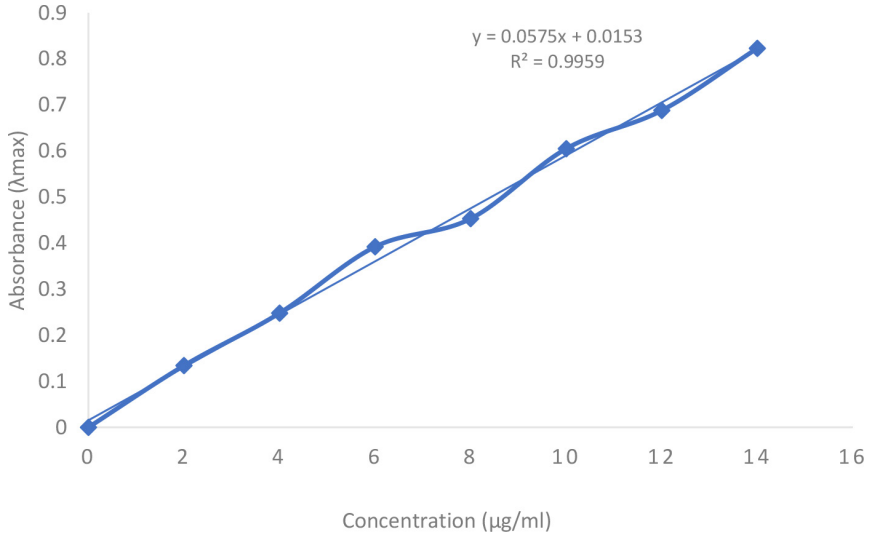


Figure 4. Standard curve of Sichuan pepper extract in Ethanol.

samples. The weight variation for the formulated jelly formulations was satisfactory, and there was no notable variation between them. The values for chewiness increased gradually, indicating that there is an increase in structural strength of the gels with an increase in the polymer amount. There was a corresponding increase in gumminess with improved internal bonding of the matrix, as shown in Table 6.

4.2.2 Thickness

The thickness of the formulated jelly formulations was fairly consistent, with negligible variation noticed between the formulations. The variation could possibly be due to the difference in pouring and solidification of the formulations.

4.2.3 pH

Evaluations on chewable jelly formulations (F1-F6) revealed that the pH level of all batches lies in the range of 6.08 to 6.38, which is relatively close to neutral. Therefore, it can be inferred that all formulations would be safe for oral administration and will not cause any form of irritation inside the mouth.

4.2.4 Viscosity

It was noted that there was a steady increase in the viscosity levels from 1085 cps in F1 to 1935 cps in F6. This indicated that the concentration of the gelling agent had been increased gradually in the formulation batches.

Table 6. Hardness, gumminess, chewiness of the prepared chewable jelly

Gumminess	Chewiness	Hardness
71± 4.6	104 ± 5.8	138 ± 6.5
86± 5.2	129 ± 6.7	162 ± 7.2
101± 5.9	147 ± 7.3	181 ± 6.8
112 ± 6.5	166 ± 6.9	205 ± 8.1
124± 7.1	182 ± 7.8	224 ± 7.6

4.2.5 Spreadability

When considering the spreadability levels, it was found that these were declining gradually from 24.8 cm/sec in F1 to 20.3 cm/sec in F6. Hence, it is quite clear that viscosity and spreadability have an inverse relationship with each other. Based on these findings, the mid-range formulations (F3-F5) seem to offer a more consistent and easily spreadable product as shown in Table 7.

4.2.6 Disintegration Test

The Disintegration Time Profile of all Jelly Formulations shown in Table 7 indicated that All Formulations had very close disintegration times (approx 21 - 24 Minutes) of all the formulations tested, F1 had the longest disintegration time due to higher levels of gelling agent resulting in more rigid matrix structures. Formulation F3 had the shortest disintegration time than F1, indicating faster disintegration.

4.2.7 In Vitro Dissolution Study

As per the findings obtained from Table 8, it can be seen that the drug release rate for all formulations (F1 to F4) increases gradually with time, which means that all the formulations have a controlled release profile. In the beginning, i.e., within the first 5 min, the release rate is lower but shows a sudden rise between the 10-min and 30-minute mark. After the 30-min mark, the rate decreases and enters a plateau region. Out of all formulations, F2 has shown the highest drug release rate (96.30%) at 40 min, followed by F1 (95.49%) and F3 (70.50%), while F1 and F2 release rates were almost similar. However, F4 showed the least release rate of 59.70%. *In vitro* release profile, cumulative release and

Table 7. The result of evaluated parameters for chewable jelly formulations

Formulation	pH ± SD	Viscosity (Cps) ± SD	Spreadability (g.cm/sec) ± SD	Weight variation	Thickness	Disintegration time
F1	6.08 ± 0.04	1085 ± 52	24.8 ± 0.7	1.46 ± 0.04	5.18 ± 0.12	24.17 ± 0.65
F2	6.14 ± 0.03	1210 ± 48	23.6 ± 0.6	1.78 ± 0.06	5.27 ± 0.14	22.83 ± 0.35
F3	6.21 ± 0.05	1375 ± 61	22.9 ± 0.5	2.21 ± 0.05	5.34 ± 0.10	21.60 ± 0.40
F4	6.27 ± 0.04	1588 ± 55	21.7 ± 0.6	2.42 ± 0.05	5.39 ± 0.13	23.07 ± 0.35
F5	6.33 ± 0.03	1760 ± 63	21.1 ± 0.7	2.63 ± 0.07	5.46 ± 0.11	22.85 ± 0.38

Table 8. *In vitro* drug release Profile

S. No.	Time (min.) F1	Percentage cumulative drug released			
		F2	F3	F4	
1	0	0	0	0	0
2	5	21.80	19.40	13.60	10.90
3	10	27.95	27.30	18.75	17.60
4	15	34.20	38.50	24.60	25.80
5	20	44.10	51.30	32.40	30.90
6	25	60.05	65.20	41.90	35.60
7	30	75.60	78.40	47.10	40.80
8	35	88.10	90.20	60.80	45.90
9	40	95.40	96.30	70.50	59.70

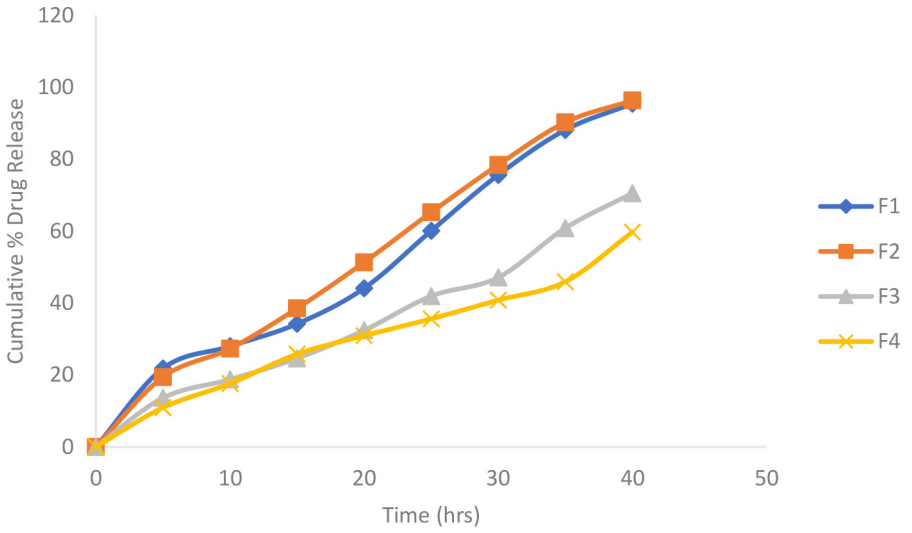


Figure 5. Zero-order release between cumulative % drug release *vs*. time.

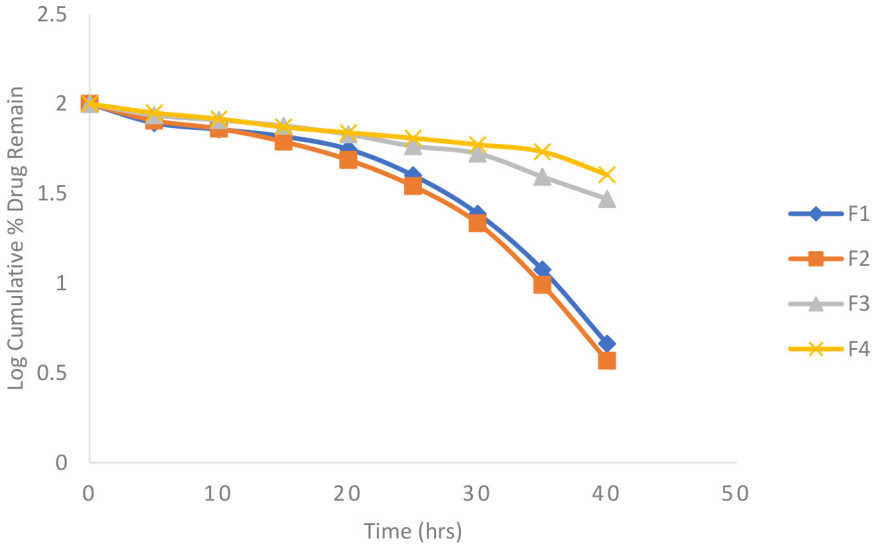


Figure 6. First-order kinetics between log cumulative % drug *vs*. time.

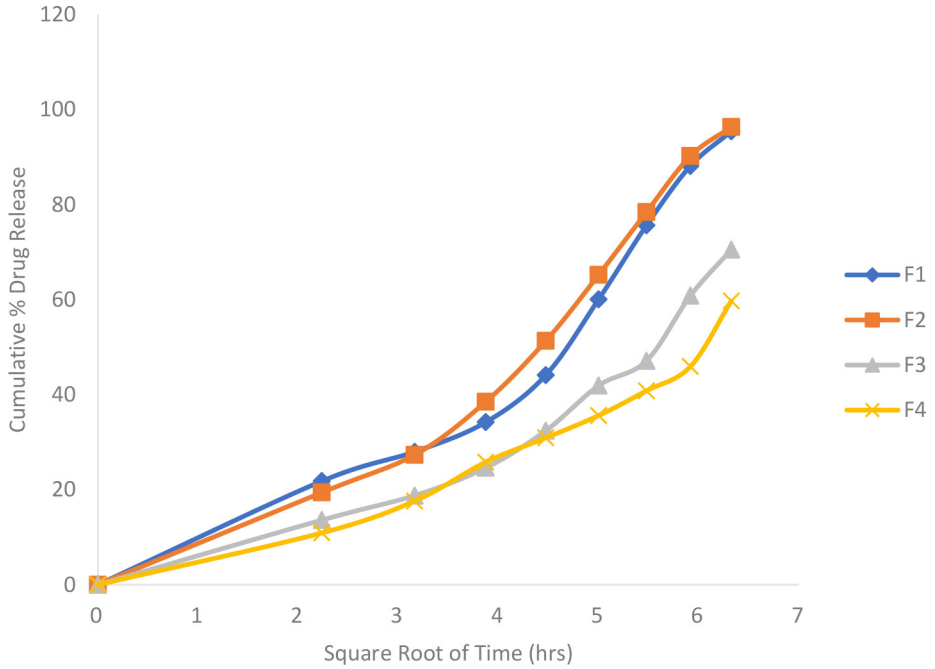


Figure 7. Higuchi release between cumulative % drug release *vs.* square root of time.

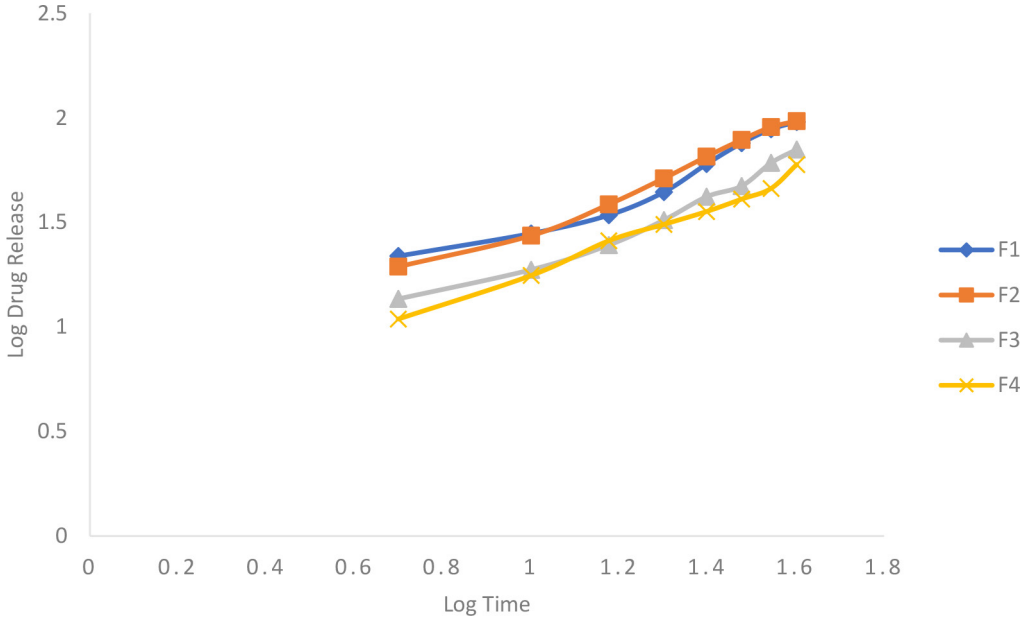


Figure 8. Korsmeyer-Peppas release between log drug release vs log time.

Table 9. The determination coefficients of different mathematical models of different formulations

Formula code	Zero order	First order	Higuchi	Korsmeyer-Peppas	n value	Mechanism of release	Best fit model
	R^2						
F1	0.9845	0.8541	0.9069	0.9382	0.62	Non-fickian	Zero order
F2	0.9937	0.8762	0.9317	0.9818	0.68	Non-fickian	Zero order

kinetics studies of prepared batches and the selected best batch of chewable jellies are shown in Figures 5- 8.

Based on the R^2 values derived from the two formulations, both had high linearity when plotted against the zero-order plot. For formulation F1, the R^2 value was recorded as 0.9845, whereas for F2, it was slightly higher at 0.9937. This implies that both formulations follow zero-order kinetics in terms of drug release. With respect to the first-order kinetic plot, the R^2 values were relatively low compared to the other models; i.e., 0.8541 and 0.8762 for formulations F1 and F2, respectively. This means that the process does not follow the concentration dependency. Regarding the Higuchi model, the linearities were 0.9069 and 0.9317 for formulations F1 and F2, respectively, implying that diffusion plays a significant role in the release process. When the Korsmeyer-Peppas model was applied to analyse the two data sets, relatively good linearity (R^2 = 0.9382 for F1 and 0.9818 for F2) was observed. It should be noted that both n values fall within 0.62 and 0.68, as shown in Table 9.

4.2.8 Anti-inflammatory Activity Test using Protein Denaturation Method

The chewable jelly sample demonstrated concentration-dependent inhibitory activity against protein denaturation, which suggests its anti-inflammatory properties. As the concentration increased, there was an increase in percentage inhibition, which shows efficient interaction of the ingredients within the formulation with the protein molecules. The IC_{50} of the formulation was estimated to be about 450 µg/ml, which implies relatively moderate inhibitory activity. On the other hand, diclofenac sodium exhibited more potent inhibitory activity with an IC_{50} of 281 µg/ml, as shown in Table 10. The relatively low activity of the chewable jelly might be due to its complex composition of various phytoconstituents.

Table 10. The result of the anti-inflammatory test of Sichuan Pepper extract and diclofenac sodium as positive control

Concentration of Sichuan pepper extract	Absorbance ± SD	% Inhibition	IC50 (ppm)
100	0.558 ± 0.009	25.6	~450
200	0.490 ± 0.010	34.7	
400	0.402 ± 0.008	46.4	
800	0.295 ± 0.009	60.7	
Concentration of diclofenac sodium (standard)	**Absorbance ± SD**	**% Inhibition**	**IC50 (ppm)**
100	0.512 ± 0.008	31.7	~281
200	0.415 ± 0.009	44.7	
400	0.290 ± 0.010	61.3	
800	0.205 ± 0.007	72.7	

Table 11. Stability parameters at different temperatures

Formula	Storage conditions (After 4 weeks)							
	25°C temperature				40°C at 75% relative humidity			
	Appearance	pH	Viscosity (cps)	Weight variation	Appearance	pH	Viscosity	Weight variation
F1	No change	6.12	1092	0.48	Slight dullness	6.05	1068	0.92
F2	No change	6.18	1245	0.52	No change	6.10	1210	0.88
F3	No change	6.25	1410	0.55	Slight stickiness	6.16	1378	1.05
F4	No change	6.30	1582	0.60	Slight dullness	6.22	1535	1.12
F5	No change	6.34	1768	0.64	Slight stickiness	6.26	1702	1.18

4.2.9 Stability Study

The formulations retained their stability after 4 weeks of storage even at room temperature. There was no significant change in appearance. pH values indicated insignificant differences and maintained neutral levels; hence, the compatibility was favourable for oral administration. Viscosity values remained unchanged, suggesting that there is no significant change in the structure of the gel formulation. Accelerated testing conditions showed some variations in some formulations. There was some dullness and stickiness, and this might have occurred because of moisture absorption. There was a little drop in pH values and viscosities, but still, these variations were minor. There was also an increase in weight variations as shown in Table 11.

5. Conclusion

This study has been able to formulate chewable jelly loaded with Sichuan pepper extract by following the process of heating and congealing in the presence of appropriate excipients such as sucrose, gelatin, citric acid, sodium citrate, and sodium benzoate. The formulations created in this study were targeted for delivery through the buccal route to overcome the problem of the first-pass effect and hence improve the bioavailability of the drugs. The physicochemical parameters of all formulations, such as pH, appearance, and viscosity, have shown satisfactory results as per the pharmacopoeia standards. Formulation F1 has performed satisfactorily among all formulations tested in this study with respect to *in vitro* drug release from the formulations. The chewable jelly dosage form also provides many more benefits such as easy dosing and patient compliance, especially in cases where the patient cannot swallow other types of dosage forms. It can be safely stored for at least three months under cold storage conditions (4°C). Thus, Sichuan pepper extract-loaded chewable jelly may prove beneficial as an alternative drug delivery system for oral ailments.

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7. References

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Shaffi Tangri: Conceptualization, supervision, methodology and editing. Vidya Marwal: Methodology, justification, writing–review and editing. Anurag Dixit: Methodology, formal analysis, writing–review and editing. Muskan Sharma: Investigation, data curation, writing–new draft. Abhijeet Singh: Formal analysis, writing–review and editing. Divyanshu Chauhan: formatting and proofreading. All authors have read and agreed to the published version of the manuscript.