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FORMULATION, DEVELOPMENT AND BIOAVAILABILITY PROFILING OF *ILLICIUM VERUM* (METHANOLIC FRACTION)

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ABSTRACT
The *Illicium verum* is an evergreen Chinese plant, from which a culinary spice named star anise is obtained and is predominantly used in Asian countries. Besides its flavor, it possesses high medicinal value. The present study was carried out to extract, phytochemical investigation, quantitative analysis, formulate and evaluate an herbal suspension containing *Illicium verum.* The total seven herbal suspension formulations were prepared by trituration method and evaluated for physical test, sedimentation volume, redispersibility, flow rate, viscosity, pH and particle size. The result of all the formulations were found to be in the range and F5 formulation showed sedimentation volume of 1.20 ml, redispersibility in 1 inversion, flow rate of 5ml/ 1 min 16 sec, viscosity of 274cP, pH of 6.67 and particle size is 20µm. All the formulations then subjected to *in-vitro* drug release studies. *In-vitro* bioavailability assessment of the suspension was performed by dialysis bag diffusion method in 7.4 pH phosphate buffer. F5 formulation released its 95 % drug in 15 minutes. Hence, the F5 formulation released the maximum drug in 15 minutes so that the bioavailability of the formulation will also be good. The *in vitro* drug release data of the optimized formulation F5 was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Higuchi's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of zero order was maximum i.e. 0.999 hence indicating drug release from formulations was found to follow zero order models kinetics. Stability study of the formulation F5 was performed for 3 months and for three different conditions like 8°C± 5°Cand 40% RH, 25°C± 5°Cand 40% RH and 40°C± 5°C and 70% RH and the result showed that herbal formulation is stable.

KEYWORDS: *Illicium verum,* Phytochemical investigation, Quantitative analysis, Herbal suspension, Trituration method, Bioavailability profiling.

INTRODUCTION
Traditional medicine system is one of the centuries-old practices and long-serving fellow human beings in fighting disease and leading a good life. The distinctive method of their traditional medicine scheme has been used by indigenous people for millennia and among the most famous are the Chinese, Indian, African medicine systems. Traditional medicine relates to any ancient and cultural healthcare practices that differ from scientific medicine and are mainly orally transmitted by groups of distinct cultures.[1] The World Health Organization (WHO) notes that assigning one definition to the diverse range of traditional medicine features and components is challenging but that a working definition is crucial. It therefore concludes that the various health methods, methodologies, understanding and beliefs of traditional medicines incorporating medications based on plants, animals and minerals, spiritual therapies, manual methods and exercises applied singularly or in conjunction to preserve well-being as well as treating, diagnosing or preventing disease.[2] Moreover, it has been stated that more than 70 percent of the population of the developing world still relies on complementary/ alternative medicine technologies, otherwise known as traditional medicine, for instance, up to 80 percent of the population in Africa, 71 percent in Chile, and 40 percent in Colombia, and others.[3,4] However, the increase in population, insufficient drug supply, prohibitive treatment costs, side effects of several allopathic drugs and the growth of resistance to presently used infectious disease medicines have resulted in enhanced emphasis on the use of plant products as a source of medicine for a broad variety of human diseases.[5,6] Approximately 121 pharmaceutical products have been developed in the last decade on the basis of traditional knowledge from different sources.[7,8] In human society from time medicinal plants have played an important role in prevention and control of diseases. The pharmaceutical suspension is a biphasic liquid or semi-solid dosage form where the finely divided insoluble solid drug particles

are homogeneously dispersed in a liquid or semi-solid medium. The insoluble solid drug particles act as the dispersed phase or internal phase. The internal phase solid particles are in the size range of 0.5- 5 µm.[9] Its uniform distribution throughout the dispersion medium or vehicle is certain by one or a combination of suitable suspending agents. The therapeutic success of any dosage form depends largely on its successful formulation of the dosage form and the bioavailability of the active medicament or drug in the site of action. It is, therefore, obvious to focus and discuss the parameters that directly influence the formulation of suspension and in turn influence the bio-availability of the drug. *Illicium verum* Hook. f. (Illiciaceae) is an evergreen, medium-sized tree with star-framed fruits, is found all through southwestern Asian countries. Other than its usage in culinary arts, star anise is one of the basic components of the Chinese traditional medicine and is by and large known for its antiviral effects. The fruits are frequently used as a well known spice in the food industry, and were also used for treatment of stomach aches and sepsis in eastern Asian traditional medicine. The phytochemical molecule shikimic acid, a significant precursor in the pharmaceutical production of the anti-influenza medication oseltamivir (Tamiflu), is derived mainly from star anise, an antiviral remedy for influenza A and B. Moreover, the same plant has produced a number of additional compounds with a variety of biological activity. Apart from its antiviral qualities, star anise has antifungal, gastroprotective, antimicrobial, antioxidant, insecticidal, anthelmintic, antinociceptive, sedative, anti-inflammatory, expectorant and spasmolytic capabilities, as well as estrogenic effects. So, aim of the studies to extract, prepare and evaluate herbal suspension formulations.

MATERIALS AND METHODS

Plant material

The medicinal plant *Illicium verum* (100 gm) was collected locally from Bhopal, M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and then in hot air oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. The leaves of *Illicium verum* medicinal plant were authenticated by a plant taxonomist in order to confirm its identity and purity.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Solvent extraction of plant extract

Coarsely powered plant parts (leaves) of *Illicium verum* (100 gm) was then extracted by successive extraction using different organic solvents; defatted with petroleum ether (40-60^{0}C) and successively extracted with petroleum ether and methanol for 36 hrs using soxhlet apparatus. To ensure complete extraction, each extract was evaporated to dryness under reduced pressure by rotary evaporator and the resulted dried residue was stored in air-tight container for further use.[13]

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures.[14,15] After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Quantitative analysis of methanolic extract of *Illicium verum*

The standard solution of anethole was prepared by dissolving 5.0 mg of drug using ethanol into 5.0 ml volumetric flask until volume of solution reach 5 ml. From the above stock solution 10ml was taken in another 100ml volumetric and volume was made up with methanol to mark and the concentration of solution become 100µg/ml. After that from the above solution the aliquots of 5-30 ml of stock solution were taken into a series of 10ml volumetric flask and volume was made up to the mark with methanol and it was analysed at λ max 254 nm using UV spectrophotometer. The standard curve was plotted between absorbance and concentration.[16] 1 gram of the extract was dissolved in methanol and was analysed at λ max 254 nm using UV spectrophotometer. The anethole content in the *Illicium verum* extract was calculated by using standard curve of anethole.

Formulation of herbal suspension

The formula for preparing 100 ml of a suspension of extracts of *Illicium verum* was as shown in Table 1. Suspension was prepared by using bioactive extract of *Illicium verum* trituration method in mortar and pestle by using the suitable suspending agent of Tween 80 and Sodium carboxy methyl cellulose (CMC) along with other excipients. One formulation was prepared without the suspending agent (CMC). The suspending agent, sodium CMC in the aqueous medium containing selected preservatives was added in mortar and pestle along with methanolic extracts of plant material with continuous triturating. Finally, by addition of purified water by continuous trituration in suspension brought up to the final volume to get the uniform product.[17,18]

Table 1: Composition of herbal suspension.

Composition	Formulations						
	F1	F2	F3	F4	F5	F6	F7
Extract (gm)	1gm	1gm	1gm	1gm	**1gm**	1gm	1gm
Tween 80 (%w/v)	0.1	0.1	0.1	0.1	**0.1**	0.1	0.1
Sodium CMC (%)	0.5	0.7	1	1.4	**1.7**	2.0	-
Methyl paraben (%w/v)	0.20	0.20	0.20	0.20	**0.20**	0.20	0.20
Lemon oil (ml)	0.01	0.01	0.01	0.01	**0.01**	0.01	0.01
Purified water (q.s.in ml)	q.s.	q.s.	q.s.	q.s.	**q.s.**	q.s.	q.s.

Evaluation of suspension formulation

Physical test of herbal suspension

The physical test of herbal formulation was carried out at room temperature (±25°C) and 45°C. The results were shown in table:

Sedimentation volume

The sedimentation volume is the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension as the suspension settles in a cylinder under standard condition. It was determined by keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain period of time and note that the volume of the sediment which is expressed as ultimate height.[18]

Redispersibility

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180º and the number of inversions necessary to restore a homogeneous suspension was determined.[17]

Rheology

The time required for each suspension sample to flow through a 10 ml pipette was determined by the apparent viscosity by using the equation.[17]

$$Flow\ rate = \frac{Volume\ of\ pippete(ml)}{Flow\ time\ (second)}$$

Viscosity

The viscosity of the sample was determined at room temperature using Brookfield viscometer at 50 rpm by using spindle no. 3.[18]

pH

The pH of suspension was determined using pH meter.

Particles size analysis

The distribution of particle size in suspension is an important aspect of its stability. Particle size distribution was carried out by using optical microscopy in dilute suspensions.[18]

***In-vitro* drug release (Bioavailability assessment)**

The in-vitro drug release study of suspension formulations were studied by dialysis bag diffusion method. Extract loaded suspension was dispersed into dialysis bag and the dialysis bag was then kept in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ±1°C throughout the experiment. During the experiment rpm was maintained at 100 rpm. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer at 254 nm. To analyze the in vitro drug release data various kinetic models were used to describe the release kinetics.

Mathematical treatment of *in-vitro* release data

The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

1. Zero-order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$$\mathbf{Q_t = Q_o + K_o t}$$

Where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution (most times, Q_o=0) and K_o is the zero order release constant.

2. First-order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution and K_1 is the zero order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its

interior, in such way, that the amount of drug released by unit of time diminish.

3. Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

The simplified Higuchi model is expressed as:

$$Q = K_H \cdot t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drug.

4. Korsmeyer-Peppas model: Korsmeyer *et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where M_t/M_∞ is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of n =0.5 for fickian diffusion and higher values of n, between 0.5 and 1.0, or n =1.0, for mass transfer following a non-fickian model. In case of a cylinder n =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent n the portion of the release curve where $M_t/M_\infty < 0.6$ should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (l) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and b value would be zero and only at^n is used. This mathematical model, also known as Power Law, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.[19-21]

Accelerated stability study

The accelerated stability study was carried out for F5formulation of bioactive constituents at 8°C, room temperature and 45°C±2 at 75%±5 humidity. The stability of herbal suspension was studied for three months. The different parameters such as pH, sedimentation volume, re-dispersibility were studied for all the formulation at 1st, 2nd and 3rd months.[17]

RESULT AND DISCUSSION

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Illicium verum* is shown in Table 2. The preliminary phytochemical screening of methanolic extract of *Illicium verum* shown that they contain Alkaloids, glycoside, steroids, proteins, flavonoids, tannins, and phenolic compounds Table 3. The amount of anethole present in the *Illicium verum* methanolic extract was found to be 42mg/gm Table 4 & Figure 1. All the prepared suspension formulations were evaluated for physical test and it found that the formulation is in liquid in nature, brown in colour, pleasant odor and suspension in texture. Sedimentation volume of all the prepared formulations was found to be in the range of 1.20 to 2.68. From the above results, it was observed that the formulation F5 was optimum and acceptable. Flow rate is the time required for each suspension sample to flow through a 10 ml pipette. The flow rate of all the formulations was found in the range of 5ml/45 sec to 5ml/1min 16 sec. The formulation F5 showed slow flow rate which is 5ml/ 1min 16 sec. Viscosity and pH of all the formulations was determined and the results were found to be in the range of 95cP to 274cP and 5.95 to 6.67pH respectively. Higher the viscosity slower will be the flow rate. As F5 showed slow flow rate so that its viscosity was found to be higher. Particle size of all the formulations observed around 16 to 22 µm Table 5. The in-vitro release of all the suspension formulations are represented in the table: From the above evaluation of all the formulations, F5 showed better in-vitro drug release and bioavailability profile Table 6 & Figure 2. In-vitro drug release of all the formulations were performed and it was found that F7 formulation which was prepared without the use of CMC (Suspending agent) is showing only 50% drug release within 15 minutes, rest all the formulations showing more than 80 % drug release within 15 minutes. So the bioavailability of the prepared formulations is

increases with the use of the suspending agent. Among them F5 formulation was found to show maximum drug release within the given period of time. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of F5 formulation showed the constant drug release from the suspension, the results of the zero order model was found to be y =6.4857x + 12.267, R2 = 0.999. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be y =-0.0829x + 2.1243, R2 = 0.9227. The Higuchi model is used to describe the limits for transport and drug release. The Higuchi model of formulation was found to be y =31.681x - 23.217, R2 = 0.984 Table 7 & Figure 3-5. Accelerated stability of selected formulation was performed for three different conditions like 8°C± 5°Cand 40% RH, 25°C± 5°Cand 40% RH and 40°C± 5°C and 70% RH and the parameters taken for the study are redispersibility, flow rate and viscosity. From the result, it showed that there were no noticeable changes in the physicochemical properties of the herbal formulation Table 8.

Table 2: Percentage Yield of crude extracts of *Illicium verum* extract.

Extract	Yield
Pet ether	0.145%
Methanol	4.759%

Table 3: Phytochemical testing of extract.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Present	Present
1.2	Mayer's reagent test	Present	Present
1.3	Wagner's reagent test	Present	Present
1.3	Hager's reagent test	Present	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent
5.	**Flavonoids**		
5.1	Shinoda's Test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
6.2	Gelatin test	Present	Present
7.	**Saponins**		
7.1	Froth test	Absent	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present

Table 4: Anethole content (mg/ml equivalent to Anethole).

Extract	Methanolic extract
Absorbance	1.473±0.025
Concentration of Anethole	42mg/gm

Table 5: Sedimentation volume, redispersibility, flow rate, viscosity, pH, and particle size of all the prepared formulations.

S. No.	Parameters	Formulations (%)						
		F1	F2	F3	F4	F5	F6	F7
1	Sedimentation Volume	2.15	2.46	1.46	2.58	**1.20**	2.68	0.54
2	Redispersibility	1 inversion	1 inversion	1 inversion	1 inversion	**1 inversion**	1 inversion	1 inversion
3	Flow rate	5ml/45 sec	5ml/57sec	5ml/ 60 sec	5ml/ 30 sec	**5ml/1min 16 sec**	5ml / 48 sec	5ml / 30 sec
5	Viscosity	150cP	205cP	185cP	95cP	**274cP**	138cP	145cP
6	pH	6.34	6.28	6.57	5.95	**6.67**	6.60	6.82
7	Particle size	17 µm	22 µm	15 µm	16 µm	**20 µm**	18 µm	28 µm

Table 6: *In-vitro* drug release of all the formulations.

S. No.	Time (Minutes)	Formulations code						
		F1	F2	F3	F4	F5	F6	F7
1	2	26.54	18.78	12.23	15.78	**25.89**	22.25	5.46
2	4	37.96	32.65	29.48	38.96	**39.45**	35.69	25.46
3	6	52.57	51.25	38.68	49.14	**50.25**	43.32	35.26
4	8	68.15	69.85	45.47	58.28	**65.13**	61.14	40.65
5	10	77.65	72.45	63.36	71.48	**77.32**	72.26	48.26
6	15	91.78	85.95	82.47	86.56	**95.78**	88.59	53.17

Table 7: Release kinetics study of F5 formulation.

Formulation	Model	Kinetic parameter values
F5	Zero Order	y= 6.4857x + 12.267 R^2=0.999
	First Order	y=-0.0829x + 2.1243 R^2=0.9227
	Higuchi	y=31.681x - 23.217 R^2=0.984

Table 8: Accelerated stability study of F5 suspension.

Parameter	Stability condition / parameter								
	8°C± 5°Cand 40% RH			25°C± 5°Cand 40% RH			40°C± 5°C and 70% RH		
Redispersibility	Good	Good	Good	Good	Good	Good	Good	Good	Good
Flow rate (ml/min)	1.15	1.14	1.125	1.20	1.18	1.17	1.01	1.11	.58
Viscosity (cp)	275	272	276	250	263	271	248	230	23

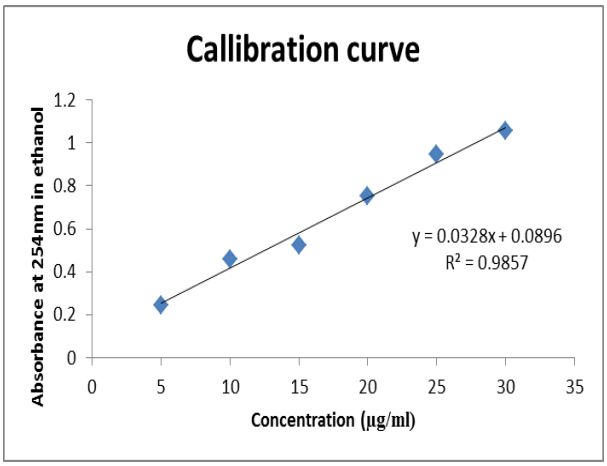

Figure 1: Calibration curve of Anethole.

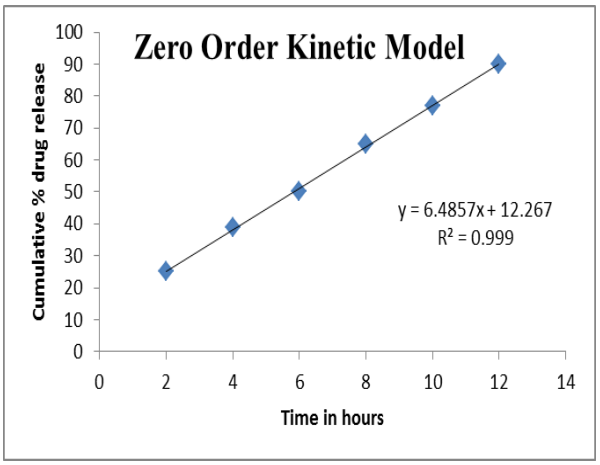

Figure 3: Zero order model of F5 formulation.

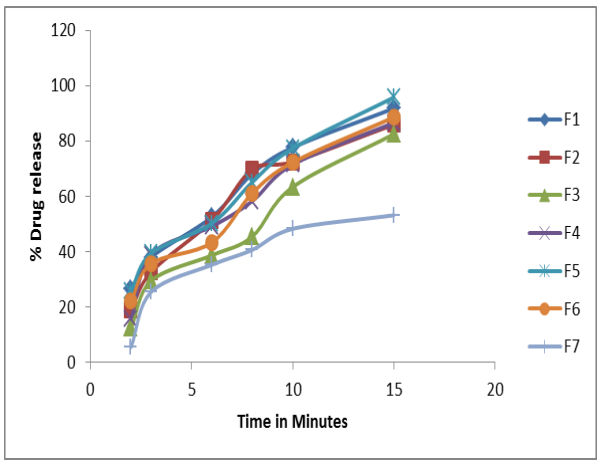

Figure 2: Cumulative percent drug releases of all formulations.

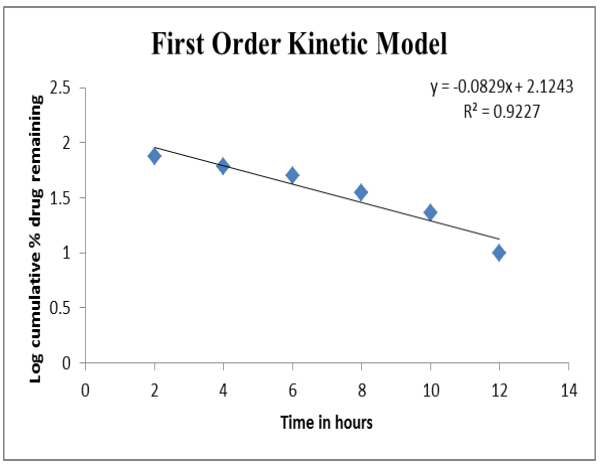

Figure 4: First order model of F5 formulation.

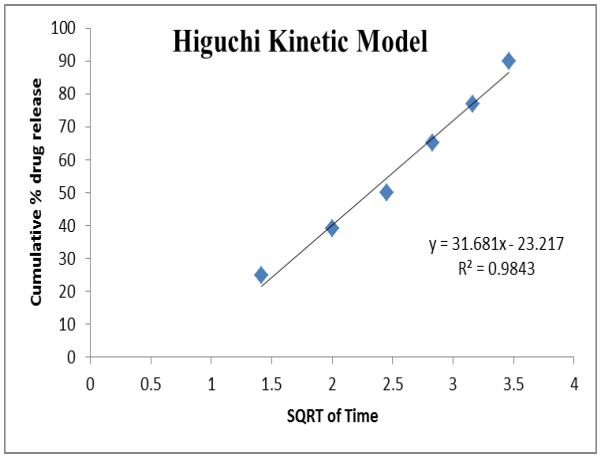

Figure 5 Higuchi model of F5 formulation.

CONCLUSION

Liquid dosage forms have the upper hand over solid dosage form in children and elder people due to them overcome the problem of swallowing. Pharmaceutical suspension is one of the most trusted and acceptable formulations among another oral dosage form because of flexibility ease of administration, easy swallowing in the administration of the drug. The herbal suspension was prepared by using lyophilized ethanolic extracts of selected plants by trituration method using a suitable suspending agent and other excipients. Herbal suspension was prepared, and stability parameters were evaluated. The prepared suspension formulation was found to have redispersibility property with sedimentation studies showed that the sedimentation volume of formulation F5, which indicates that the formulation was optimum and acceptable. There was no significant change observed in physicochemical and organoleptic behavior. Among all the formulations F7 was prepared without the bioavailability enhancer and rest all the formulations were prepared with the use of bioavailability enhancer. The *in-vitro* bioavailability assessment of the F5 formulation observed the 95 % released of the drug in 15 minutes as compared to the F7 formulations, which indicates that it has the increased bioavailability of the formulation.

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