DEVELOPMENT AND EVALUATION OF HERBAL CAPSULE FORMULATIONS FOR THERAPEUTIC TARGETS OF HYPERLIPIDEMIA

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ABSTRACT

Hyperlipidemia is a term used to describe a group of genetic and acquired disorders for high lipid levels in the body. Several non-herbal medications are available to treat hyperlipidemia but they have several drawbacks. Herbal remedies have been made available for a variety of ailments by traditional systems. Hence, in the present study, three different herbal capsule formulations were prepared from the aqueous extract of Rasona, Tvak, Arjuna, Guduchi, and Mesasringa using a wet granulation technique and evaluated using different parameters. The dissolution profile of prepared capsules showed better drug release of 64.05%, 71.16%, and 76.14% in phosphate buffer (pH 6.8) for C1, C2, and C3, while 53.69%, 59.33%, and 62.08% drug release in 0.1N HCl within 2 hours. The presence of allicin and E-Ajoene in C1, Cinnamonaldehye, Eugenol, and Arjunic acid in C2, and Berberine, Dolichandroside A, and

Chrysin-7- rutinoside as an active constituent in C3 formulation was analysed by HPTLC study. The nutritional value of C3 showed 86.14%, 9.33%, and 1.92% for carbohydrates, protein, and cholesterol respectively. A stability study confirmed no presence of heavy metals, moisture content, and microbial contamination in these formulations before and after 6 hours. Consequently, based on these findings, the polyherbal capsule made from these plant extracts was found to be safe and effective for the treatment of lipidic diseases as well as may improve patient compliance.

Keywords- Hyperlipidemia, Soxhlet extraction, Rasona, Tvak, Arjuna, Guduchi, Mesasringa, Capsules.

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INTRODUCTION

A set of lipid metabolic diseases known as hyperlipidemia (HLP) are characterised by an increased synthesis or a prolonged degradation of atherogenic lipoprotein molecules. Additionally. It has been noted as a possible risk factor for cardiovascular diseases (CVDs)[1,2]. Nearly 523 million cases of CVD were reported, and 18.6 million individuals died from these in 2019[3]. The HLP causes atherosclerosis, cardiac, cerebrovascular, and peripheral vascular disease[4]. Among all, diabetes mellitus and atherosclerosis are considered major complications of HLP[5]. Various factors responsible for this condition include diet, hypertension, obesity, pregnancy, diabetes, AIDS, anorexia nervosa, alcohol & smoking, acute hepatitis, and nephrotic syndrome[6].

Pathophysiology of hyperlipidemia

The liver and intestines play significant roles in lipid metabolism[7]. It involves two pathways: exogenous and endogenous (Fig. 1). The exogenous route is the initial stage in the metabolism of dietary lipids. In the endogenous pathway, chylomicrons are responsible for the transport of dietary lipids, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL). They are essential in the metabolism of endogenously produced lipids[8]. Whereas, in HLP conditions, increasing triglyceride (TG), cholesterol (CHOL) levels, and fluctuating elevated LDL may also be accompanied by a decline in HDL levels[9].

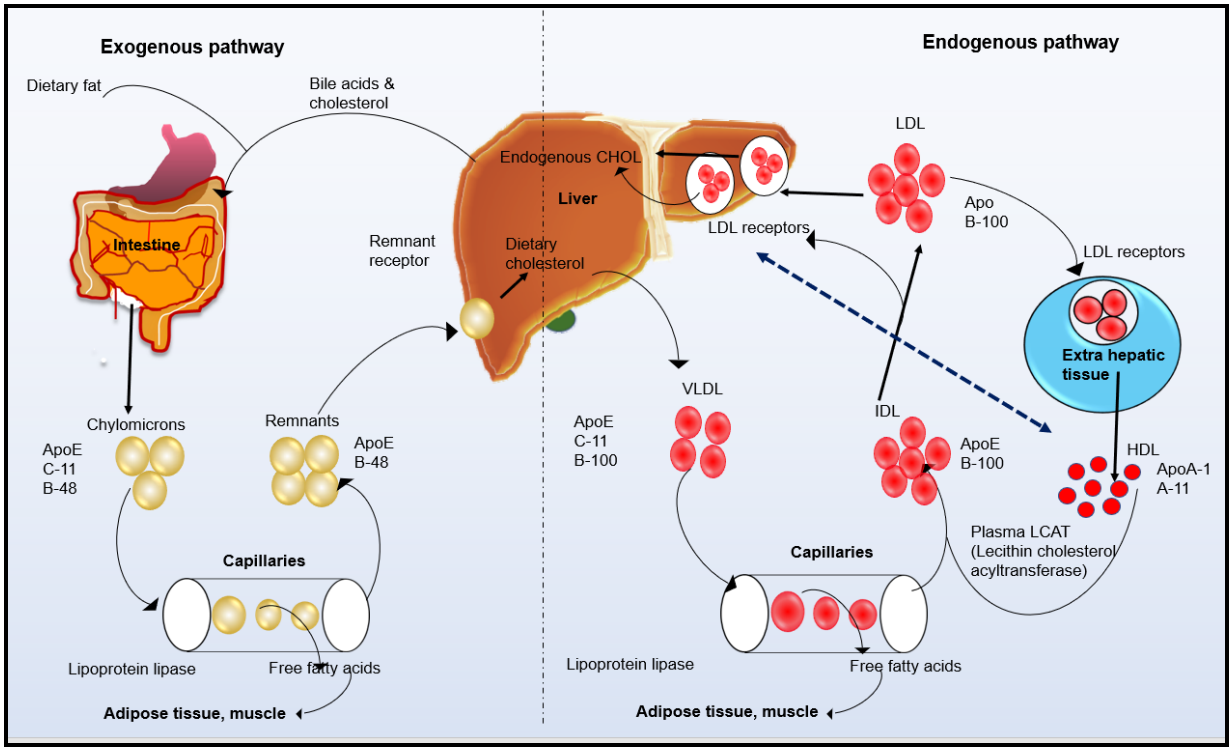


Fig. 1: Metabolism of lipoproteins[8] (Own creation).

Several treatment options are available to treat HLP using non-herbal therapeutics e.g., statins, bile acid sequestrants, fibric acid derivatives, niacin, etc. However, there are some drawbacks and side effects of these treatments that impact individuals' health. "Ayurveda" (a traditional medicinal system), can provide a suitable approach to explore the herbal formulation for managing HLP to combat these consequences. Ayurvedic medicine aims to improve mental, physical, and spiritual health of well-being. Keeping a proper balance of all vital energies, the body's physical degradation and sickness processes can be halted[10]. Polyherbal formulations have demonstrated greater efficacy than the action of a single herb and can better treat various severe ailments. Capsules are more reliable in delivering these formulations as they replenish essential drugs, healthy nutrients, minerals, and vitamins. In addition to improving bioavailability, making them suitable for clinical studies. Convenient packaging enables uniform dose, mobility, and consumer compliance. They also mask undesirable tastes and odours and provide rapid drug release[11].

Therefore, in this investigation, the plant extracts from the rasona, tvak, arjuna, guduchi, and mesasringa were used to formulate polyherbal capsules to reduce the symptoms of HLP more safely and effectively. Table 1 specifics the description of selected pants. The capsules were prepared by wet granulation and then tested for microbial analysis, disintegration, IVRT, and stability study. Overall, this study demonstrates the formulation and evaluation of polyherbal capsules to address the issue of HLP.

Table 1: Pharmacognostic accounts of selected plants.

Rasona	Garlic, Lahsan	Mature bulb	*Allium Sativum Linn*	Liliaceae	Hepatoprotective, anti-inflammatory, antidiabetic.	12,13
Tvak	Dalchini	Dried internal bark	*Cinnamomum zeylanicum Blume*	Lauraceae	Antioxidant, antidiabetic, antihyperlipidemic.	14
Arjuna	Arjuna	Dried stem bark	*Terminalia arjuna*	Combretaceae	Anti-microbial, antifeedant, cardioprotective.	15,16
Guduchi	Giloya, Gulvel	Stem, root, leaves	*Tinospora cordifolia*	Menispermaceae	Anti-diabetic, antihyperlipidemic, anticancer.	17
Mesasringa	Medhshing	Whole plant	*Dolichandrone falcata*	Bignoniaceae	anti-inflammatory, anxiolytic, antioxidant.	18

MATERIALS AND METHODS

Below section deals with the materials and methods utilised for the current research.

Materials

Starch was availed from Thermo fisher scientific, India for this research. Merk life sciences, Mumbai procured MCC pH 102, and Silica gel G-60-F254 was purchased from E. Merck, India. Hydrochloric acid and Sodium chloride were obtained from Loba Chemie, Mumbai. Spectrum chemicals and Otto Chemie Pvt Ltd, Mumbai supplied Talc & Eudragit® E100 respectively. All chemicals and reagents were used of an analytical grade.

Methodology

1. Collection and authentication of plant material

The selected plants (Rasona, Tvak, Arjuna, Guduchi, and Mesasringa) were collected and dried under the shade. The herbarium of these plants was prepared and authenticated.

2. Preparation of aqueous extracts for selected plants

The Soxhlet extraction technique was used to prepare the extracts. For extraction, 20 gm of crushed, dried *A. sativum* bulbs were placed in a thimble. Distilled water (DW) used as extracting solvent was added to a flask with a spherical bottom that was connected to the heating mantle. As soon as the solvent evaporated, it travelled to the condenser. Condensate drops were collected into a reservoir. The cycle was restarted when the solvent was reintroduced into the flask after going through the syphon. The operation was carried out for 16 hours to complete the extraction. The product was collected, purified, filtered, and dried. Repeated the same procedure for *C. Zeylanicum* bark, *T. Arjuna* stem bark, *T. Cordifolia* dried stem, and *D. Falcata* leaves[19–23].

2.1 Preliminary phytochemical tests

Phytochemical screening is an essential parameter in identifying herbal plants' active components. Various phytochemicals can be identified using the following tests (Table 2)[24].

Table 2: Tests for preliminary screening of phytochemicals.

Alkaloids	Wagner’s test	Brown precipitate (ppt).
	Dragendroff”s test	Reddish brown ppt.
	Hager’s test	Yellow ppt.

	Mayer's test	Cream or white-colored ppt.
Glycosides	Keller-Killiani test	A pale blue color layer.
	Borntrager's test	The formation of red color.
	Ninhydrin test	Blue colour.
Proteins	Molisch's test	Purple or rosy violet shading appeared at the intersection of the two fluids.
Carbohydrates and sugars	Fehling's test	brick-red ppt.
	Benedict's test	Red colour ppt.
Phenolic compounds & Tannins	Extract + a few drops of 5% alcohol+ $FeCl_3$ solution	Bluish black (or) dark green colour.
Flavonoids	Shinoda's test	Red colour.
Steroids	Salkowski test	Blue-green-colored solution.
Saponin	Foam test	The formation of a 1cm foam layer.
Fixed oils and Volatile oils	--------	A tiny amount of the extract was squeezed between two filter sheets. Both oily spots on the sheet and oil evacuation at RT revealed the existence of fixed and volatile oil, respectively.

2.2 Evaluation of quality control parameters for selected plant extracts

The evaluation was done using various parameters for each extract, including organoleptic like colour, odour, and taste[25]. The percent yield of obtained aqueous extracts (AEs) was calculated by using the following formula[26] (1), and the loss on drying (LOD) was calculated by equation[27] (2).

$$Yield(\%) = \frac{Weight\ of\ dry\ extract\ obtained}{Weight\ of\ dry\ part\ used} X\ 100 \qquad (1)$$

$$LOD\ (\%) = \frac{Wt.of\ sample\ before\ drying - Wt.of\ sample\ after\ drying}{Wt.of\ sample\ before\ drying} X\ 100 \qquad (2)$$

Approximately 2 gm of a chosen plant's extract was precisely weighed and placed in a crucible that had been tared, lighted, and heated to 350°C for 1 hour, progressively raised the temperature to 550°C and maintained for 5 hours in a muffle furnace. Dried extracts were dispersed, & weighed and total ash content was calculated by equation[28] (3).

$$Total\ ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} X\ 100 \qquad (3)$$

The acid-insoluble ash content was determined by recently acquired ash when heated for 5 min in 25 mL of HCl and calculated using equation[28] (4). Whereas total ash was boiled for 5 min with 25 cc of water to determine the amount of water-soluble ash[29] (5).

$$Acid\ insoluble\ ash\ (\%) = \frac{Weight\ of\ acid\ treated\ ash}{Weight\ of\ sample} X\ 100 \qquad (4)$$

$$Weight\ soluble\ ash\ (\%) = \frac{Initial\ weight\ of\ ash - Mass\ of\ water\ insoluble\ residue\ in\ total\ ash}{Weight\ of\ sample} X\ 100 \qquad (5)$$

The water-soluble extractives (determined using 100 ml of chloroform water) and alcohol-soluble extractives (determined in 100 ml of the alcohol) with 5.0 grams of chosen plant extract for each. The results were obtained using equations (6) & (7) respectively[30].

$$Water\ soluble\ extractive\ (\%) = \frac{Initial\ mass - Mass\ of\ water\ soluble\ extraction\ residue}{Initial\ mass} X\ 100 \qquad (6)$$

$$Methanol\ soluble\ extractive\ (\%) = \frac{Initial\ mass - Mass\ of\ alcohol\ soluble\ extraction\ residue}{Initial\ mass} X\ 100 \qquad (7)$$

To evaluate the micromeritic properties of dried plant extracts, bulk density (BD) and tapped density (TD) were performed by graduated cylinder method as per the WHO standards. The BD and TD were determined by the equation[31] (8), (9)[32] respectively.

$$Bulk\ density = \frac{Weight\ of\ extract}{Volume\ of\ extract\ in\ measuring\ cylinder} \qquad (8)$$

$$Tapped\ density = \frac{Weight\ of\ extract}{Tapped\ volume\ of\ extract} \quad (9)$$

The pH of various extracts of chosen plants was measured by a glass electrode pH meter[27]. Heavy metals in the obtained extract, like lead, arsenic, cadmium, and mercury was detected using atomic absorption spectrophotometry (AAS). The test solution was prepared in a dry Kjeldahl flask. Weighed 2 g of chosen plant's sample, added suitable nitric acid, and then heated continuously till the solution was colorless. The sample was then transferred to a 25 ml volumetric flask, and the volume was made-up with DW. A similar process was used to create a blank solution but without sample[33]. The standard solutions of the elements to be examined were prepared separately as well as the absorption maxima of the blank and each reference solution were measured individually[34].

The presence and absence of pathogens in the prepared extract were determined using an inoculating loop. A portion was streaked from the enrichment culture on the surface of the agar medium. The dishes were covered and placed in an inverted position, and incubated. According to the ayurvedic pharmacopoeia, the color of colonies was observed for the presence of pathogens within the samples.

E. coli: MacConkey agar medium; brick red colonies- presence of *E. Coli*.

Salmonella spp.: Bismuth sulphite agar; black- green color- presence of *Salmonella spp.*

S. Aureus: Vogel-Johnson agar; Black color colonies- presence of *S. Aureus.*

Pseudomonas aeruginosa: Cetrimide Agar; Greenish color colonies existence of *P. aeruginosa*[35].

2.3 Chromatographic study profile with marker compound

Each plant's 10 µL extract solution and a standard solution of 40 µg/mL were applied on an aluminium plate pre-loaded with Silica gel 60-F254 using an applicator (Linomat-IV). The plates were developed in a glass tank using a mixture of chromatographic solvents for selected plant materials as mobile phase at RT (28±2℃). The composition of the mobile solvents was optimised to achieve good separation. After air drying, the plate was derivatised with anisaldehyde H_2SO_4 and heated at 105°C in the oven for 5 min[36].

3. Formulation of polyherbal capsules

3.1 Granulation of solid dry extract of selected plants

Granulation was achieved using the wet granulation technique. A 10% (w/w) Eudragit® E100 binder was employed to produce granules. Each solid dry extract (SDE) was weighed separately and pulverised in an acetone solution containing 12.5 w/v Eudragit E. This combination was carefully mixed until it reached the desired consistency and filtered through a sieve with a minimum opening of 1 mm. The granules were dried for 2 hours in an oven at 25 °C before being screened and stored. Obtained granules was named G1, G2, G3, G4, and G5 for the selected plants[37].

3.2 Preformulation study of plant extract granules

Particle size and angle of repose

The symmetry of particle size is regarded as a critical characteristic for achieving the optimal efficacy of the therapeutic constituent. Each plant granule was processed through separate sieves, mesh # 40 and 60, to ensure particle size uniformity[38]. An angle of repose can employ the flow property of the materials and be calculated using equation[39] (10).

$$\theta = \tan^{-1}(h/r) \qquad (10)$$

Where,

h = pile height, and r = pile radius

Fourier transform infrared (FTIR) spectroscopy

The FTIR of each plant granule was carried out individually for qualitative compound identification. One mg of the dried extract granules was pressed with 10 mg of potassium bromide (KBr) pellets. The mixture was loaded in an FTIR spectroscope (Agilent Technologies Cary 650 FTIR) and scanned with a range from 500 to 4000 cm^{-1} with a 4 cm^{-1} resolution[40].

3.3 Polyherbal capsule formulation

Capsule 1: Drug Allium sativum

Accurately weighed 1 kg of *A. Sativum* buds were crushed and formulated as capsules using the formula represented in Table 3. The obtained formulation was sticky in nature and difficult to fill in the capsule; therefore, it was kept for drying in a shade till the moisture content was reduced to 10%. The 300 capsules of size 1 were filled using hand filling capsule machine for each formulation and 8 batches were evaluated for physical appearance, fill weight, and disintegration time[41].

Table 3: Formula for batch preparation of capsule 1.

		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1.	Garlic paste	100	100	100	100	100	100	100	100
2.	MCC pH 102	200	190	300	90	150	240	195	110
3.	Starch	190	200	90	300	240	150	195	280
4.	Talc	10	10	10	10	10	10	10	10

Capsule 2: Drug C. Zeylanicum and T. Arjuna

Followed the above procedure for formulation of capsule 2 *(C. Zeylanicum* and *T. Arjuna* mixed in a 1:1 ratio). Table 4 represents the 8 different formulation batches of capsule 2[41]:

Table 4: Formula for batch preparation of capsule 2.

		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
	Zeylanicum & T. Arjuna	150	150	150	150	150	150	150	150
1.	MCC pH 102	170	140	200	150	119	100	260	60
2.	Starch	170	200	140	190	150	260	100	300
3.	Talc	10	10	10	10	10	10	10	10

Capsule 3: Drug T. Cordifolia and D. Falcata

Followed the above procedure for formulation of capsule 3 (*T. Cordifolia* and *D. Falcata* mixed in a 1:1 ratio). Table 5 represents the data for 8 different formulation batches of capsule 3[41].

Table 5: Formula for preparation of capsule 3.

		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1.	*T. Cardifolia & D. Falcata*	150	150	150	150	150	150	150	150
2.	MCC pH 102	170	140	200	150	119	100	260	60
3.	Starch	170	200	140	190	150	260	100	300
4.	Talc	10	10	10	10	10	10	10	10

3.4 Evaluation of capsules by using different parameters

The developed formulations were evaluated by following tests.

Weight variation test

The test was executed to estimate the quantity of powder in each capsule. From each capsule formulation, namely capsules 1, 2, and 3, twenty herbal capsules were randomly selected and weighed accurately. Following that, the average weight was taken and compared to the individual weight. The % weight variance was computed following USP (2010) Standards. The herbal capsule must be between 90% and 110% of the weight of each unit, as estimated theoretically[42].

Disintegration test

The disintegration test estimates the time for particular capsules to break down into particles large enough to pass through a 10-mesh screen at a given temperature (37 ºC±2 ºC) and pH. A total of 3 herbal capsules were selected randomly from batches of capsule formulations. The capsule was loaded in each tube and then suspended in beakers containing simulated gastrointestinal fluid (SGF, pH 1.2) for 30 minutes. The best batch from the experiment was identified for the dissolution experiment[43].

In-vitro drug release (IVRT) study

The IVRT study of selected batches of herbal capsules was accomplished by the USP dissolution test apparatus (Electrolab dissolution tester) employing a basket stirrer (Method 1). The two solvents (PBS-pH 6.8 and 0.1 N HCl) were agitated at 50 rpm and thermostated at 37±0.5 ℃. An aliquot of the sample (1 mL) was pipetted from the release media at specific intervals (15, 30, 45, 60, 90, and up to 120 minutes) and replaced with an equivalent quantity of the fresh dissolution media to ensure the sink condition. The samples were assayed under UV (UV- 1800, Shimadzu, Japan) at respective λmax[44].

Determination of moisture content and heavy metals

The test was performed using the Karl Fischer instrument. The water content of the capsules was maintained by keeping them at a constant temperature (15–25°C) and RH (45–55%). Because low RH causes capsules to become brittle, while high RH causes flaccidity, additionally, excessive moisture may interact with encapsulated substances and affect their stability[45]. The presence of heavy metals in the prepared formulation was carried out using AAS. The complete procedure is described in above section.

Microbial analysis of prepared formulation

Sample preparation

One gm of the fine capsule powder was suspended in 19 ml of normal saline, and each was thoroughly mixed via a swirling (5-10 minutes). To remove the residues of the raw material, the resultant solution was centrifuged at a speed of 2000 rpm at room temperature (RT). The supernatant was then used for further analysis[46].

The microbial analysis was accomplished by the most convenient pour plate method, and the supernatant's total viable count (TVC) was calculated. In this approach, one ml of the acquired residue was added to sterilised saline solution (SSS) (9 ml) in screw-capped containers and shaken to produce 10^1 dilutions. To prepare 10^2 dilutions, one ml of the initial dilution was added to another SSS (9 ml). Further, the dilutions were conducted up to 10^6 times. About 20–25 ml of molten soybean casein digest agar was added to each petri plate aseptically and plates were incubated for 48 hours, then incubation of colonies was counted as well as reported[46].

Determination of nutritional value

Nutritional value can be determined by estimating the carbohydrate, protein and cholesterol concentration in formulations. The procedure is described below in Table 6.

Table 6: Estimation of nutritional value.

Stock solution	1 g of each capsule powder + 10 ml of HCl	1 g each capsule powder + 100 ml DW	0.1g each capsule powder + 10 ml isopropanol
Standard	Glucose	Bovine serum	Cholesterol
Reagent	Anthrone	A: 2% Na_2CO_3 in sodium hydroxide B:0.1% Rochelle salt & 0.5% $CuSO_4$ (1:1) C: Reagent A (49 ml) + Reagent B (1ml) D: Folin Ciocalteau (FC): DW (1:1)	$FeCl_3$ reagent, followed by conc. H_2SO_4
Optical density (nm)	630	660	540
R.T. (min)	10	10	10
References	[47]	[48]	[49]

Chromatographic study

For the detection, the most sophisticated automated device (CAMAG LINOMAT V) was utilised for the chromatographic analysis. The percolated aluminium plates (10 × 10 cm) with Silica Gel 60 F254 were utilised, with a 0.2 mm thickness. Before chromatography, the plates were pre-washed with methanol and activated at 60 °C for 5 min. Accurately weighed 1 g of finished product equivalent to 1 g of extract was taken in a separate iodine flask. Then 50 ml methanol was added into each flask and refluxed for 1 hour. Filter the solution. Then the filtrate was concentrated into 1-2 ml. This solution was utilised for HPTLC fingerprinting. The plates were analyzed at 366 and 254 nm using CAMAG Scanner-3 and LINOMAT-V, and recorded the Rf value of each[50].

Stability study

To evaluate the stability of the formulation, an accelerated stability study was conducted as per ICH Q1A standard (R2) standards. Optimized capsules were packed separately in suitable packaging and kept in a stability compartment at 40 ± 2 ℃ and 75 % RH for 6 months. The granules were evaluated by estimating heavy metals, microbial analysis, nutritional value, and drug release[51].

RESULT AND DISCUSSION

1. Collection and authentication of plant material

Rasona, Tvak, Arjuna, Guduchi, and Mesasringa were collected and dried under the shade. The herbarium of these plants was prepared and identified by expert Dr Mohan Waman, Principal and HOD, Department of Botany, Jaipur.

2. Preparation of aqueous extracts for selected plants

The aqueous extracts of selected plants were prepared using the Soxhlet extraction method. Dried aqueous extracts were stored and further evaluated.

2.1 Preliminary phytochemical tests

The phytoconstituents were identified by using various chemical tests indicated in Table 7 below:

Table 7: Phytochemical screening of selected plants.

Alkaloids	+	-	+	+	+
Glycosides	+	+	+	+	+
Saponins	+	-	+	+	+
Tannins & phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroids	+	+	-	+	-
Fixed oils	+	+	+	-	-
Carbohydrates	-	+	+	+	+
Proteins	+	-	+	+	+

(+) = Present; (-) = Absent

Preliminary phytochemical analysis was done to identify the phytoconstituents (PCs) in selected plants. It showed that, Rasona bulbs: presence of all the PCs except the carbohydrates, Tvak bark: absence of PCs like alkaloids, saponins and proteins. In contrast, Arjuna bark: absence of steroids. The stem of Guduchi: absence of fixed oil, while Mesasringa leaves: absence of steroids and fixed oil. The presence of these PCs possesses therapeutic and physiological activities.

2.2 Evaluation of quality control parameters for extract

The following Table 8 indicates the results obtained from the quality assessments of selected plant extracts.

Table 8: Quality assessment of plant extracts.

Organoleptic study					
Colour	Off-white with pink shading	Brown	Greenish grey	Yellow tint	Green
Odour	Distinctive	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Pungent (Spicy Flavour)	Aromatic	Bitter Astringent	Sweet, Bitter, Pungent Astringent	Bitter
LOD & Ash value					
LOD (%)	5.03	22.50	3.12	3.27	2.69
Total ash (%)	0.63	3.59	19.31	4.87	4.65
Acid insoluble ash (%)	0.23	1.21	0.78	1.54	1.29
Water soluble ash (%)	0.43	22.81	1.59	0.73	0.21
Extractive value					
Water soluble	20.10	10.37	45.04	23.86	13.22
Methanol soluble	6.01	15.21	40.19	12.65	10.92
pH	5.7	5.1	6.0	5.07	5.04
Micromeritic properties					
Bulk density (g/cm^3)	0.242	0.238	0.361	0.252	0.233
Tapped density (g/cm^3)	0.244	0.236	0.359	0. 248	0.231
Heavy metals					
Lead	-	-	-	-	-
Arsenic	-	-	-	-	-
Cadmium	-	-	-	-	-

Mercury	-	-	-	-	-
Microbial contamination					
TVC	-	-	-	-	-
Specific Pathogens					
E. Coli	ND	ND	ND	ND	ND
Salmonella spp	ND	ND	ND	ND	ND
S. Aureus	ND	ND	ND	ND	ND
P. aeruginosa	ND	ND	ND	ND	ND

Based on the above-mentioned findings, it was determined that organoleptic evaluation demonstrated accurate extract identification, extractive and ash values proved quality and purity, low LOD indicated minimal microbial growth, and no extracts were contaminated with any pathogens, heavy metals, or microbes. Because of this, the quality control test was successful and was considered for the final formulation development.

2.3 Chromatographic study profile with marker compound

The results of chromatographic profile are mentioned below (Table 9, Fig. 2).

Table 9: HPTLC of selected extract.

1.	*A. Sativum*	Allicin and E ajoene	Formic acid: Toluene: Ethyl acetate (0.1:19:1)	254
2.	*T. Arjuna*	Arjunic acid	Ethyl acetate	254
3.	*C. Zeylanicum*	Cinnamonaldehye and Eugenol	Toluene: Formic acid: Ethyl acetate (19: 0.1:1)	366
4.	*T. Cordifolia*	Berberine and Tinosporaside	Butanol: acetic acid: ethyl acetate: DW (3:1:5:1)	366
5.	*D. Falcata*	Lutelion and dolichandroside-A	Ethyl acetate: Acetic acid (5:0.5)	366

The HPTLC profiles of plant extracts showed various active chemical constituents present in them. Also, it indicated that extract was derived from a genuine plant or parts of the plant.

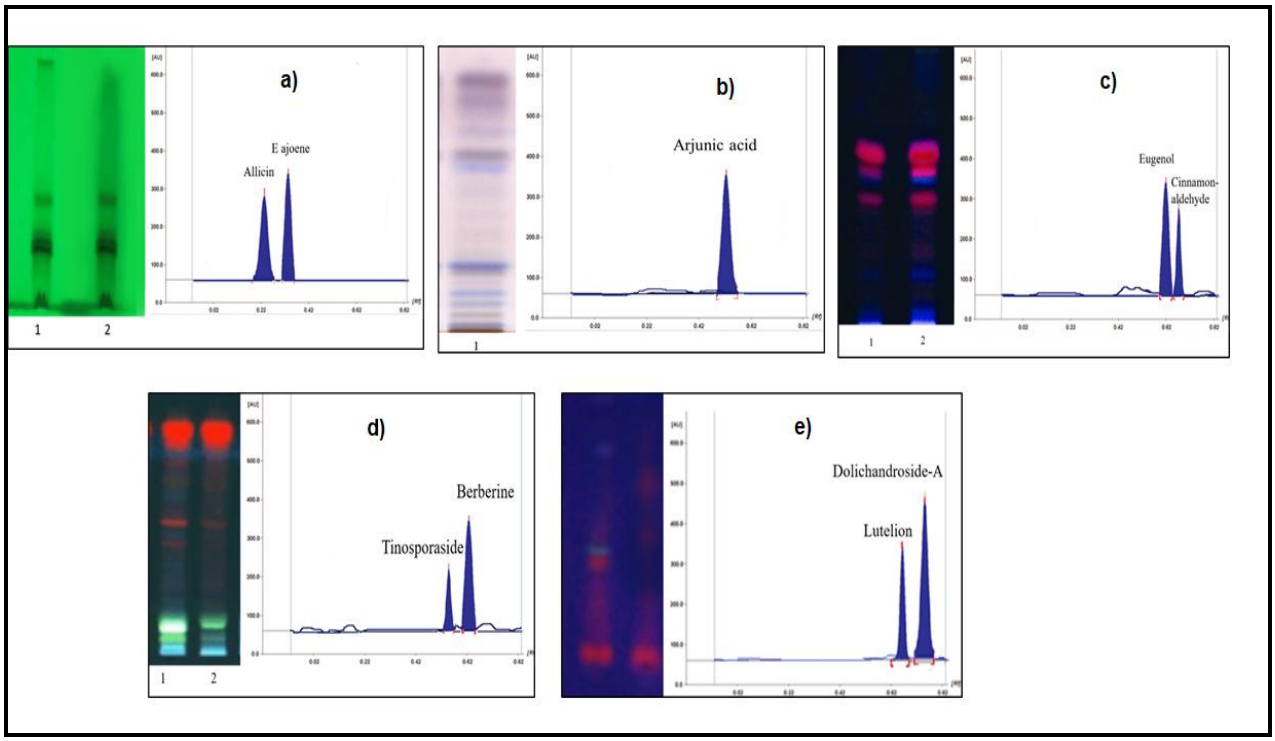


Fig. 2: HPTLC of a) *A. Sativum* b) *T. Arjuna* c) *C. Zeylanicum* d) *T. Cordifolia* e) *D. Falcata.*

3. Formulation of polyherbal capsules

3.1 Granulation of solid dry extract of selected plants

Granulation was achieved using the wet granulation technique. The prepared granules of extract were stored and evaluated using the following parameters.

3.2 Preformulation Study

The study was carried out to determine the physicochemical properties of granules. The data obtained from the study is mentioned in following Table 10.

Table 10: Preformulation evaluation.

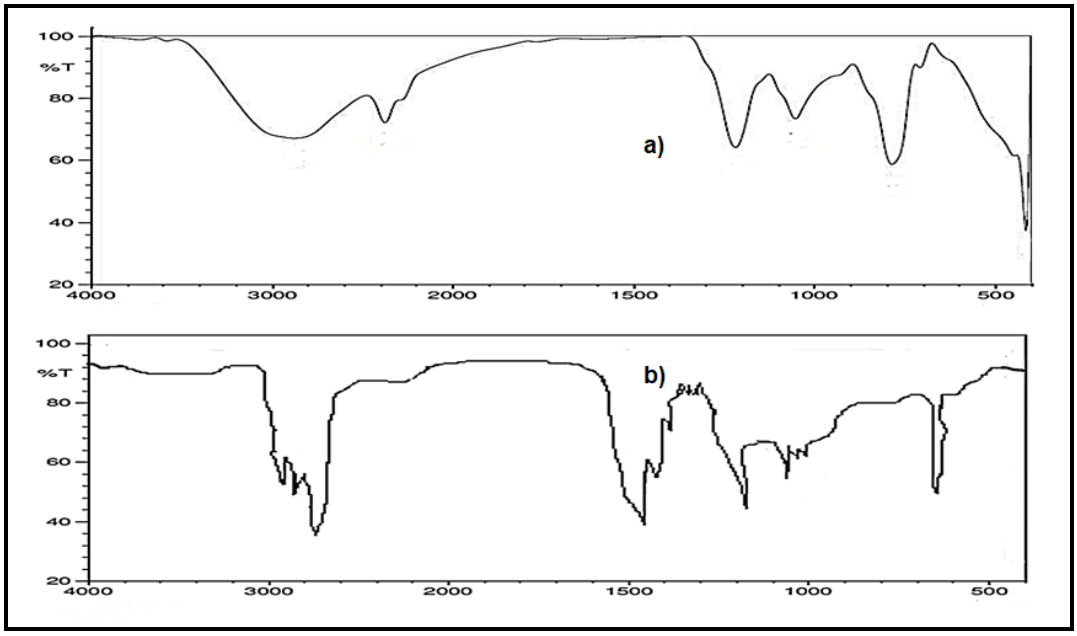
Particle size (µm)	595	355	592	353	593
Angle of repose	24.58°	24.39°	24.48°	24.36°	24.56°

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of granules with different excipients was recorded between 4000-500 cm^{-1} (Table 11, Fig. 3).

Table 11: FTIR values of plant extract granules.

1.	***A. Sativum***	1899.9	Bending vibrations of C-H
		1619.47	C=C Alkene
		1395.62	O-H carboxylic
2.	***C. Zeylanicum***	1463.87	Bending vibrations of CH_3
		2924.97	stretching vibrations of C-H
		3470.93	Stretching of O-H
3.	***T. Arjuna***	1715.43	C=O stretching vibrations of carboxylic acid
		1465	Bending vibrations of C-H
		3317.2, 3817.5	O-H stretching
4.	***T. Cordifolia***	2560	S-H thiol stretching
		1618.64	C=C of conjugated alkene
		1633.06	C=C of cyclic alkene
5.	***D. Falcata***	3585.64	O-H stretching
		1995.79	C=C=C stretching



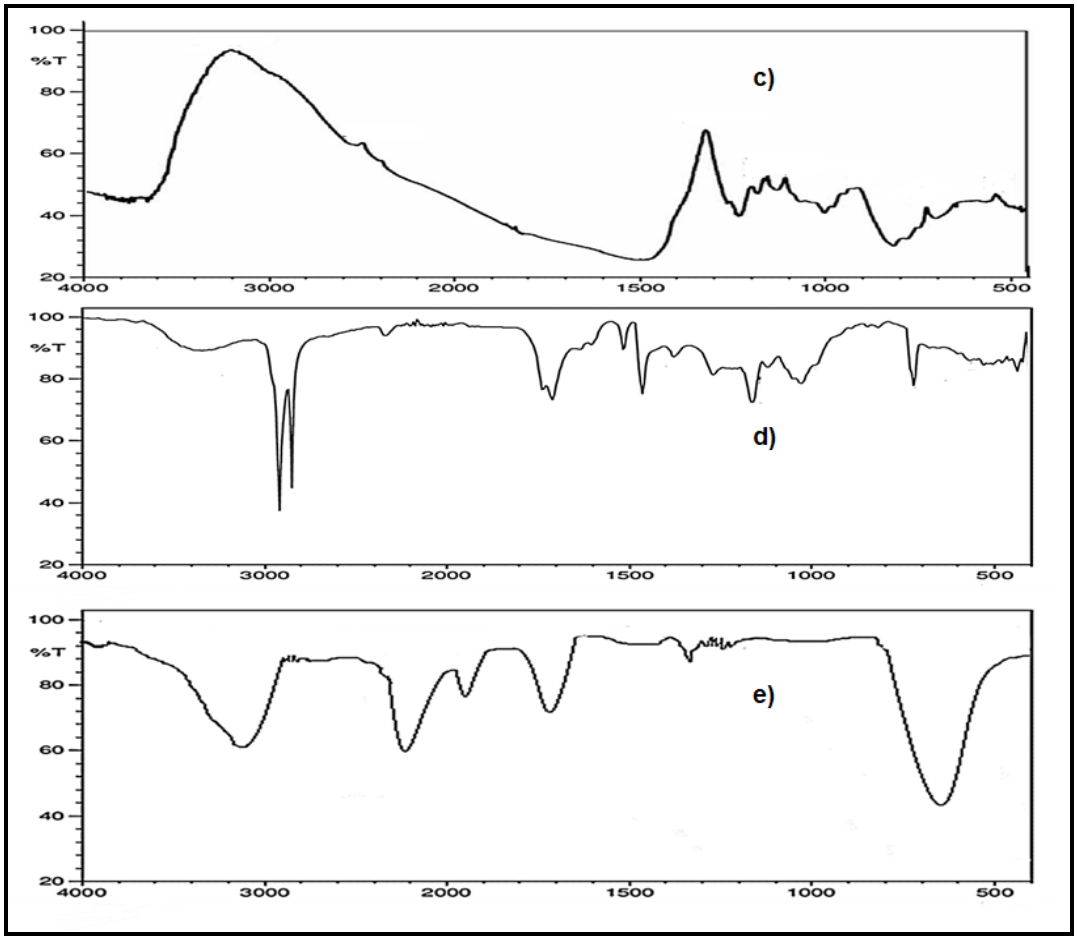


Fig. 3: FTIR profiles of granules of a) *A. Sativum* b) *C. Zeylanicum* c) *T. Arjuna* d) *T. Cordifolia* e) *D. Falcata*.

The significant peaks indicated that O-H, C=C, C-H, C=O, S-H, and CH_3 functional groups were present in selected plant extracts. Hence, the FTIR study confirmed a reliable and sensitive method for detecting biomolecular composition.

3.3 Polyherbal capsule formulations

Three different capsules were formulated: one was *A. Sativum* containing capsule, the second was the combination of two extracts of *C. Zeylanicum* and *T. Arjuna*, and the third was a combination of *T. Cordifolia* and *D. Falcata*. Eight batches of each capsule were prepared, and among these, the best batch was selected and evaluated.

3.4 Evaluation of capsules by using different parameters

Weight variation test

Among the 8 batches of three different capsules, batches F7, F1, and F4 from capsules 1, 2, and 3 respectively passed the test of weight uniformity within the 90-110% acceptance criteria given in USP. Hence, these 3 batches were selected for further study (Table 12).

Disintegration (DT) study

All eight batches of three capsules were subjected to the disintegration test. Batch F7, F1, and F4 from capsule 1, capsule 2, and capsule 3 showed better results, and DT for F7, F1, and F4 was found to be 11, 9, & 9 min respectively (Table 12). These three batches were selected for further study.

Table 12: Weight variation and DT profile for all three formulation batches.

Capsules	***Weight variation test (%)***							
1	89.23	87.69	84.56	89.82	83.66	82.152	**95.62**	88.20
2	**109.23**	114.12	112.89	119.87	117.56	112.25	112.78	117.44
3	87.66	85.52	83.23	**91.25**	88.91	86.45	88.21	86.89
DT study (min)								
1	12.30	15.34	21.26	12.56	13.45	14.30	**11**	12.5
2	**09.00**	11.23	10.45	9.58	25.14	10.43	12.34	11.22
3	10.37	12.53	13.10	**09.00**	12.56	9.34	13.26	11.23

In-vitro drug release (IVRT) study

The IVRT was accomplished on capsules selected from the best batches, which gave better results in F7, F1, and F4 are named C1, C2, and C3, respectively and the drug release profiles for these capsules were conducted in two different media, PBS-pH 6.8 and 0.1N HCl, for 2 h. The present study explored that the % drug release profile for the selected capsules from best batches C3 shows higher drug release in both the media than C1 and C2 (Fig. 4).

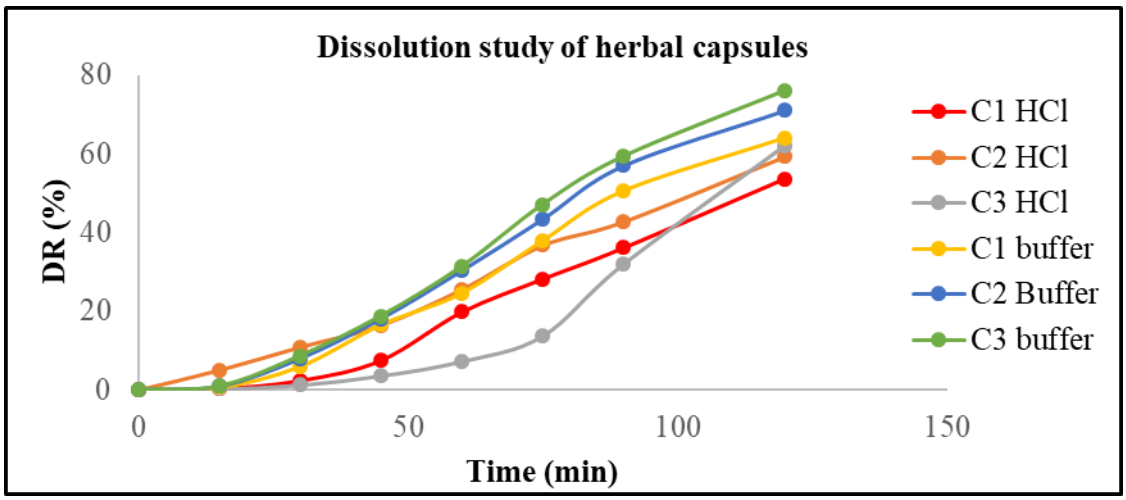


Fig. 4: IVRT of herbal capsules.

Determination of moisture content, heavy metals, and nutritional values

The result showed no change (NC) in the appearance and properties of capsules due to the effect of parameters like temperature and humidity. Also, the complete absence of heavy metals and microbial contamination in all three capsules (Table 13).

Table 13: Data for moisture content, heavy metals, microbial analysis & nutritional values.

Moisture content			
Temperature (15-25℃)	NC	NC	NC
RH	NC	NC	NC
Heavy Metals			
Lead	-	-	-
Arsenic	-	-	-
Cadmium	-	-	-
Mercury	-	-	-
Microbial Analysis			
Total viable count (TVC)	Nil	Nil	Nil
Nutritional values (%)			
Carbohydrate	84.56	85.32	86.14
Protein	8.29	9.21	9.33
Cholesterol	2.32	2.31	1.92

Chromatographic study for capsules

Results obtained from the HPTLC profile finished products developed from selected plant extracts are given below (Table 14, Fig. 5).

Table 14: HPTLC of finished products.

Capsules 1	Allicin, E-ajoene	0.22, 0.36

Capsules 2	Cinnamaldehyde, Eugenol, Arjunic acid	0.42, 0.65, 0.75
Capsules 3	Berberine, Dolichandroside A, Chrysin-7- rutinoside	0.36, 0.43, 0.79

The HPTLC fingerprint of 3 different capsules showed the present of active constituents with R_f values matched with the standard value. The presence of this important bioactive in final formulations makes them pharmacologically valuable.

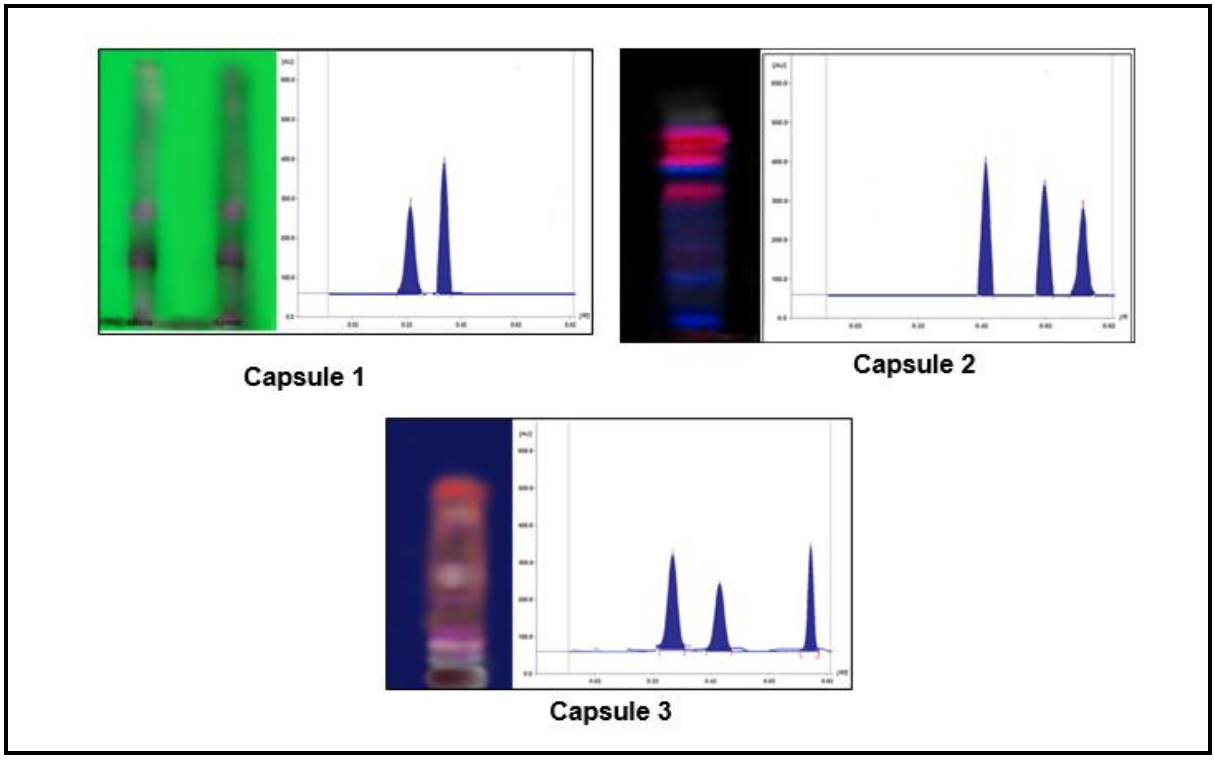


Fig. 5: HPTLC of polyherbal capsules.

Stability study

The stability of the formulations was rechecked after 6 months. The results indicated that no heavy metals were detected, absence of microbial contamination, and no change was observed in the nutritional values of all three capsules (Table 15). Apart from that, the herbal formulation did not show any significant change in the drug release (%) after 6 months (Fig. 6).

Table 15: Stability study after 6 months.

Heavy Metals			
Lead	-	-	-
Arsenic	-	-	-

Cadmium	-	-	-
Mercury	-	-	-
Microbial Analysis			
Total viable count (TVC)	Nil	Nil	Nil
Nutritional values (%)			
Carbohydrate	84.56	85.32	86.14
Protein	8.29	9.21	9.33
Cholesterol	2.32	2.31	1.92

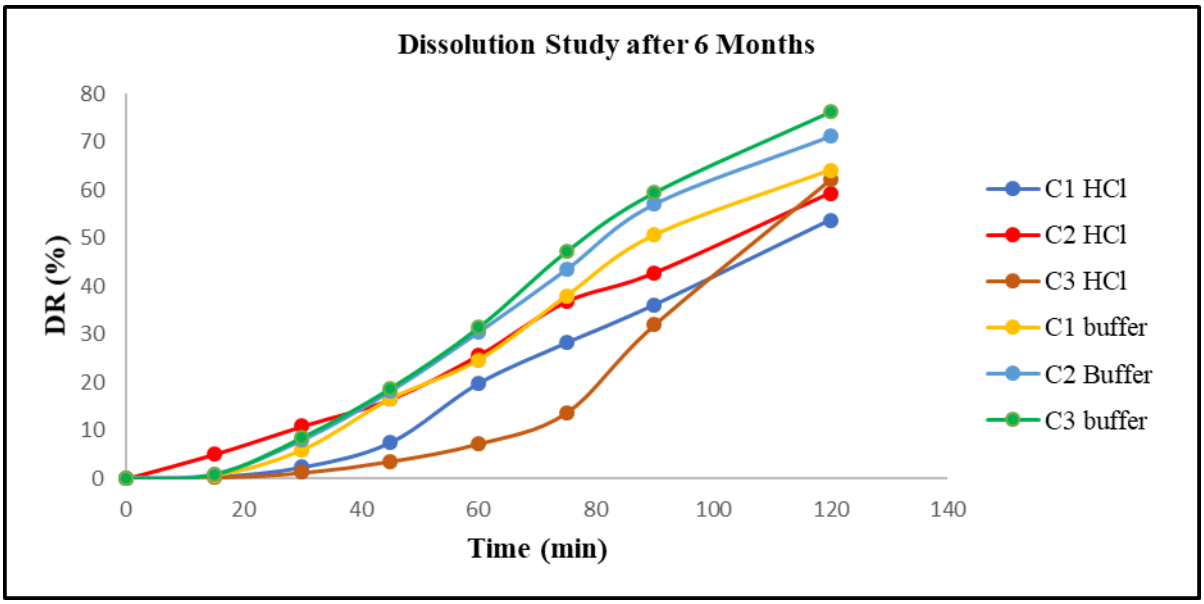


Fig. 6: IVRT after 6 months.

CONCLUSION

In the era of the 21st century, Hyperlipidaemia and Diabetes Mellitus are considered the main culprit to developing cardiovascular ailments. By 2030, it is expected that this will be the leading global cause of death and impairment. Therefore, it should be treated effectively. The current target-based synthetic drugs available for treatment are not fulfilling the need for safe and long-term therapy. Hence, traditional medicines (Ayurveda) are gaining attention of many researchers. Ayurveda uses the tridosha principle in understanding diseases and therapies. Secondly, increasing the acceptability of formulations and creating critical scientific data regarding the traditional system of medicine will go a long way in promoting them. Therefore, in the present investigation, the antihyperlipidemic formulations were successfully prepared using 5 different plant extracts and evaluated. Formulations showed better physicochemical properties of all 3 capsule formulations such as dissolution and disintegration profiles with no detection of heavy metals or pathogens.

The study concluded that the prepared formulations provide long-term, safe, and effective treatments for hyperlipidemia with no side effects. Fortunately, due to the incorporation of improved quality control and regulatory measures, it is foreseen that herbal medicine will soon be integrated into conventional medical systems, as the “age of herbs is about to come.”

Conflicts of interest

The author declares no conflicts of interest.

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