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Poly-herbal tablet formulation by design expert tool and in vitro anti-lipase activity

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Abstract

Background: Traditional medicine being ethnic is preferred worldwide even in these modern days. Obesity is a lifestyle disorder. Many chemically synthesized medicines are available. Poly-herbal medicines can be one of the safest alternatives with less side effects in treating obese patients.

Results: The in vitro anti-lipase activity was carried out for a different concentration. The formulation of the poly-herbal tablets was designed using the Design Expert software. The pre-compression and post-compression studies show that the formulation F6 showed better results of all the formulations designed. Stability study results showed that the poly-herbal tablets were stable throughout the studies.

Conclusion: The results show that F6 is the better formulation based on the tablet evaluation, and all the extracts showed inhibitory activity against pancreatic lipase indicating its active role in the treatment of obesity.

Keywords: Poly-herbal tablet, In vitro anti-lipase activity, Design expert tool

Background

Obesity is a severe as well as chronic disorder which is also an important risk factor for lifestyle-related chronic disease. When an imbalance is caused in the consumption of energy (food) and expenditure of energy leads to the condition called obesity, lack of knowledge regarding the nutritional diet and large consumption of fatty materials leads to their accumulation in the human body. The fats when consumed in excess get stored in the adipose tissue of the body. This accumulation leads further to other chronic diseases and disorders in human beings and acts as the risk factor. Secondary diseases are hypertension, type II diabetes, coronary heart disease, hyperlipidemia, and many more disease and disorder. Obesity is said to be one of the considerable contributors of non-communicable chronic disease worldwide.

The increase in consumption of high-fat food and the modern lifestyle are the most prevailing reasons for obesity. Obesity is becoming a global problem in developed as well as developing countries. According to the WHO (World Health Organization), the prevalence of obesity nearly tripled between 1975 and 2016. In 2016, about 13% of the world population was found to be obese. In the USA, the obesity prevalence is 26% which is the highest and 3% in South East Asia which is the lowest. Three hundred forty million children and adolescents are overweight and obese in 2016 [1, 2]. The key enzyme responsible for hydrolysis of fats is pancreatic lipase (PL) which is found in the GI tract; this enzyme can also be considered as the crucial target in lipid metabolism and absorption [3].

The imbalance of energy expenditure and product remains the primary influencer of obesity; other factors like psychotropic treatment steroid hormones contraceptives protease inhibitors also contribute to weight gain that is drug-induced weight gain.

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Decrease in physical activities of the human being with the past lifestyle is also the cause of obesity. Chemicals such as poly-chlorinated bi-phenols and some alkyl phenols act on the endocrine system as system disruptors and affect the normal function leading to obesity [4].

The time of body intake and the number of meals taken vary from person to person and sometimes community. The time of food intake also plays a key role in the balancing of energy consumption, and these directly influence obesity [5].

The breaking down of the dietary fats and oils is carried out by the enzyme lipase (triacylglycerol acylhydrolases). This enzyme has been synthesized by the higher organisms [6].

The inhibition of pancreatic lipase activity which reduces intestinal absorption is one category of drug, and orlistat is a good example. The appetite-suppressing mechanism is used by other categories of drugs, and the example is sibutramine. Apart from providing anti-obesity, these drugs are responsible for having different side-effects like headache, increase in blood pressure, dry mouth, constipation, and many others.

As the chemically synthesized drugs have many side effects, the demand is towards herbal or natural anti-obesity medicine. Many medicinal valued herbs have been studied for anti-obesity activity by preparing extracts of different solvents [7, 8].

Methods

Materials

Indian elm (*Holoptelea intigrifolia*), kodo millet (*Paspalum scrobiculatam*), myrobalan (*Terminalia chebula*) are collected from the local region of Belagavi (India) located at 15.87° N 74.5° E and authenticated at ICMR Belagavi by Dr. Harsha Hegde.

Collection and extraction

All the raw/crude materials were dried at room temperature for 7 days. The dried raw materials were ground to form a coarse powder. Cold maceration was carried out for the coarse powder using 70% ethanol. The extract was filtered after 48 h of cold maceration. Marc was used for further extraction with the Soxhlet apparatus using 95% ethanol. Filtrates collected from both cold maceration and Soxhlet were combined together. Combined filtrate is then taken from concentration using a rotary evaporator (IKA RV 10) at 40 °C under reduced pressure. The concentrated extract was from the rotary evaporator and then finally kept on water bath to evaporate the remains of solvents in the extract.

Phyto-chemical investigations

The phyto-chemical investigation was carried out for all crude drug materials for the identification of different classes.

Physico-chemical investigation

Ash value

Total ash Weigh about 2–5 g of raw material and then take it into the crucible and keep it at a temperature of about 500 °C–600 °C in the muffle furnace till the raw material forms carbon-free ash, then it is cooled, weigh the ash formed, and calculate using the formula:

$$\text{Total ash (\%)} = [\text{weight of the total ash obtained}/\text{weight of the crude drug}] \times 100$$

Acid insoluble ash The carbon-free ash obtained is boiled with about 25 ml of hydrochloric acid for about 5 min. The insoluble matter is collected in the crucibles (sintered) with ashless filter paper and then wash with hot water and ignite at 500 °C till constant weight is obtained and further calculate.

$$\text{\%Acid insoluble ash} = [\text{weight of acid insoluble ash}/\text{weight of the crude drug}] \times 100$$

Water soluble ash The ash (carbon-free) obtained is boiled with 25 ml of water; then with ashless paper, it is collected in the crucibles (sintered), washed with hot water, and ignited at 500 °C to obtain constant weight; the crucible is weighed and calculation is done.

$$\text{\%Water soluble ash} = [(\text{weight of total ash} - \text{weight of water insoluble ash})/\text{weight of crude drug}] \times 100$$

Extractive values

Alcohol soluble extractives About 4 g of raw material is added to 25 ml of alcohol in a flask and is kept for 24 h aside. The solution was filtered and the filtrate is poured in petri plates and kept at 105 °C for 5–6 h, and finally, the extract is weighed.

$$\text{\% Alcohol soluble extractive} = [\text{weight of extract}/\text{weight of plant material}] \times 100$$

Water soluble extractives About 4 g of raw material is added to 25 ml of water in a flask and is kept for 24 h aside. The solution was filtered and the filtrate is poured

in petri plates and kept at 105 °C for 5–6 h, and finally, the extract is weighed.

$$\%\ \text{Water soluble extractive} = [\text{weight of extract/weight of plant material}] \times 100$$

Loss on drying (LOD) Weigh about 2 g of raw material and add it to a porcelain dish and keep it in an oven at a temperature of about 100–105 °C until a constant weight is gained and then cooled and weighed.

$$\text{LOD(Loss on drying)} = \text{Initial weight of the raw material} - \text{Final weight of the raw material}$$

In vitro lipase activity

The plant extracts were first macerated with phosphate buffer saline (1 mg/ml) for 4–5 h at 37 °C, and then the solution is centrifuged at speed of 3500–4000 rpm for 10–15 min. The supernatant is further used as the stock solution, and further working standards are prepared by diluting with the phosphate buffer saline. Standard solution is prepared by dissolving one capsule content of orlistat in 12 ml of dimethylsulphoxide (DMSO) solution. The enzyme solution is prepared by dissolving 6 mg of porcine pancreatic lipase enzyme in 10 ml of the buffer solution (should be prepared and used freshly). Both the standard and the sample (plant extract) are incubated at 37 °C after adding 50 μl of enzyme solution, 25 μl of PNPB (8.4 μl of PNPB in 10 ml of acetonitrile) solution, and 100 μl of buffer solution. ELISA plate reader is used to determine the lipase activity at 400 nm and the inhibition percentage was calculated by using the formula:

$$\text{Percentage inhibition} = [(\text{absorbance of blank} = \text{absorbance of test})/\text{absorbance of blank}] \times 100$$

Anti-oxidant activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) reagent is used for performing the anti-oxidant activity by UV-spectroscopy method. DPPH solution of 0.1 Mm concentration is prepared to water as a solvent. Different concentrations of the sample solutions are prepared from a stock of 1 mg/ml stock. Ascorbic acid is used as the standard reference substance and the stock solution of 1 mg/ml is prepared. An equal amount of DPPH is added to an equal amount of both the sample and standard solution separately and allowed to react in a dark environment, and then these solutions are checked for absorbance at 517 nm under UV-spectroscopy. The process is carried out in triplicate and the average is considered.

$$(\%)\text{DPPH Scavenging Effect} = [(\text{Absorbance of standard} - \text{Absorbance of sample})/\text{Absorbance of standard}] \times 100$$

Design expert DoE

The 3^2 full factorial design was applied to the formulation design of the poly-herbal tablets. The two different factors are evaluated in this design at three different levels. The two independent variables selected are ethyl cellulose (binder) and microcrystalline cellulose (disintegrant). There are three different levels for the selected variables as low, intermediate, and high, and they are coded as − 1, 0, and + 1, respectively. The responses are considered as dependent variables, and they are hardness, friability, and disintegration time of the designed and formulated poly-herbal tablets. The software Design Expert 12 (Stat-Ease Minneapolis, MN, USA) was used for the design of the formulation. The total of 9 runs (formulations) were designed (Table 1) and the relationship of the dependent and independent variables was studies by gaining the surface responses, and finally, the significant model was achieved.

1. Independent levels:
   Ethyl cellulose(X1)
   Microcrystalline cellulose (X2)
2. Dependent levels:
   Hardness (Y1)
   Friability (Y2)
   Disintegration time (Y3)

Table 1 Levels of independent variables in the tablet design

Codes	Code levels		Actual values (mg)	
	X1	X2	X1	X2
F1	+ 1	+ 1	40	40
F2	0	− 1	30	20
F3	− 1	0	20	30
F4	+ 1	− 1	40	20
F5	0	0	30	30
F6	− 1	+ 1	20	40
F7	+ 1	0	40	30
F8	0	+ 1	30	40
F9	− 1	− 1	20	20

Table 2 Formula table

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Indian elm	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Kodo millet	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Myrobalan	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Ethyl cellulose	40 mg	30 mg	20 mg	40 mg	30 mg	20 mg	40 mg	30 mg	20 mg
Microcrystalline cellulose	40 mg	20 mg	30 mg	20 mg	30 mg	40 mg	30 mg	40 mg	20 mg
Magnesium Stearate	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
Stevia	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg
PEG-4000	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Dibasic calcium phosphate	q/s	q/s	q/s	q/s	q/s	q/s	q/s	q/s	q/s
Total weight	420 mg	420 mg	420 mg	420 mg	420 mg	420 mg	420 mg	420 mg	420 mg

Tablet formulation

Pre-compression studies

Angle of repose The angle of repose study was carried out for the powder by the funnel method. Blend of powder was taken and poured using the funnel to form a heap of the blend. The funnel was adjusted so that the tip of the cone heap just touches the lower tip of the funnel. The base diameter of the blend heap and the height of the heap were measured and the same was carried out three times, and the average diameter was calculated. Further, the angle of repose was calculated using the formula:

$$\text{Tan}\,\theta = \text{height of the cone formed by powder} / \text{radius of the cone formed by powder}$$

Loose bulk density The bulk density of the powder is measured by filling the graduated cylinder with the powder of known quantity. The volume of the graduated cylinder is measured and density is calculated by using formula:

$$\text{Bulk Density} = \text{mass of powder} / \text{volume of the powder in graduated cylinder}$$

Tapped bulk density Density tester apparatus is used to carry out the tapped density parameter. The known mass of powder is filled in the graduated cylinder of the density tester and then fixed to the apparatus. The testing apparatus is tapped from a specific height of 14 mm and another 3 mm on the surface of the density tester for 100 taps or until a constant volume is measured, and then tapped density is calculated by:

$$\text{Tapped Density} = \text{mass of powder taken} / \text{final volume of powder obtained after tapping process}$$

Carr's compressibility index The compressibility is calculated by using the results obtained from bulk density and tapped density and then substituting them in the formula:

$$\text{Carr's Compressibility Index} = 100\left(V_i - V_f\right)/V_i$$

where V_i = initial volume of the powder before tapping process and V_f = final volume of the powder after tapping process.

Hausner ratio Hausner ratio is the value obtained from the initial volume of the powder to that of the final volume of the powder after the tapping process.

$$\text{Hausner ratio} = \mathrm{V_o}/\mathrm{V_f}$$

where V_0 = unsettled apparent volume of the powder and V_f = final volume of powder after tapping.

Formulation of tablets

The plant extracts and the excipients are weighed in accordance to the formula designed by the help of the DoE software, and the formula is shown in Table 2. The ingredients are mixed properly and sieved to obtain uniform mixture of the ingredients. The mixture is then

Table 3 Phytochemical investigation data

Test	Indian elm	Kodo millet	Myrobalan
Alkaloids	+	+	–
Carbohydrates	–	+	–
Flavonoids	+	–	–
Tannins	+	+	+
Steroids	–	–	–

Table 4 Physico-chemical investigation of extract

Parameters	Indian elm	Kodo millet	Myrobalan
Lod (loss on drying)/moisture content	28%	22%	23%
Total ash	11%	2.5%	2.5%
Acid insoluble ash value	2%	1%	1%
Water soluble ash value	1.5%	1.5%	1.5%
Alcohol soluble extract	7.5%	10.75%	17.7%
Water soluble extract	37.5%	13.25%	19.5%

taken to tablet press, and the tablets are formed by direct compression technique.

Post-compression studies

All the post-compression study triplicates were recorded, and the average was considered as the final record.

Hardness The hardness of the tablets is measured using the Monsanto hardness tester, and the hardness of the tablets is recorded in kg/cm^2 unit.

Thickness The thickness of the tablets is measured using vernier calipers, and the thickness is recorded in mm unit.

Diameter Diameter is measured in mm unit by using the vernier calipers, and the data is recorded.

Friability Roche friability tester is used for performing the friability studies of the tablets. Twenty tablets from each batch are taken and weighed together and recorded as the initial weight of the tablets. Then the tablets are loaded in the apparatus and the apparatus is rotated for 4 min that is 100 rotations (25 rpm). Finally, the tablets are removed and de-dusted. The de-dusted tablets are weighed and recorded as final weight, and friability is calculated by:

$$\text{Percentage of Friability} = (\text{Initial weight} - \text{final weight})/\text{Initial weight} \times 100$$

Weight variation For studying the weight variations in the tablet formulation, twenty tablets were taken from each bath and weighed individually and the weight was noted. The average weight of the tablets was calculated and then further substituted in the formula

Weight variation = individual weight of the tablet/average weight of the tablets × 100

Disintegration The disintegration of the tablets was carried out using the disintegration apparatus, 900 ml of distilled water is added to the disintegration vessel, and six tablets from each batch are taken and loaded in the apparatus. The temperature is maintained at 37 ± 2 °C. And 28–32 cycles per minutes frequency is adjusted, and the time taken for the tablets to disintegrate is recorded and the time taken must not be more than 15 min for the conventional tablets.

Accelerated stability testing

The stability of the formulated poly-herbal tablets is carried out for the period of 30 days at 25 °C ± 2 °C/RH 60 ± 5% (room temperature) and 40 °C ± 2 °C/RH 75 ± 5% (accelerated temperature); the evaluation is performed on the 7th, 15th, and 30th days.

Results

Phyto-chemical investigation results

Phyto-chemical investigation results are shown in Table 3.

Results of Physico-chemical investigation

Results of Physico-chemical investigation are shown in Table 4.

Results of in vitro anti-lipase activity

Results of in vitro anti-lipase activity are shown in Table 5 and Fig. 1.

Table 5 In vitro lipase activity results

Concentration (μg/ml)	10	20	40	80	160	320
Orlistat	2.249489	103.8855	284.6626	489.7751	497.955	565.2352
Formulation	7.770961	98.77301	185.2761	393.2515	492.0245	520.2454
Indian elm (HI)	32.92434	50.30675	148.8753	363.3947	400	497.7505
Kodo millet (PS)	37.01431	103.8855	179.1411	233.5378	401.0225	492.0245
Myrobalan (TC)	30.87935	81.39059	135.5828	211.8609	424.3354	496.319

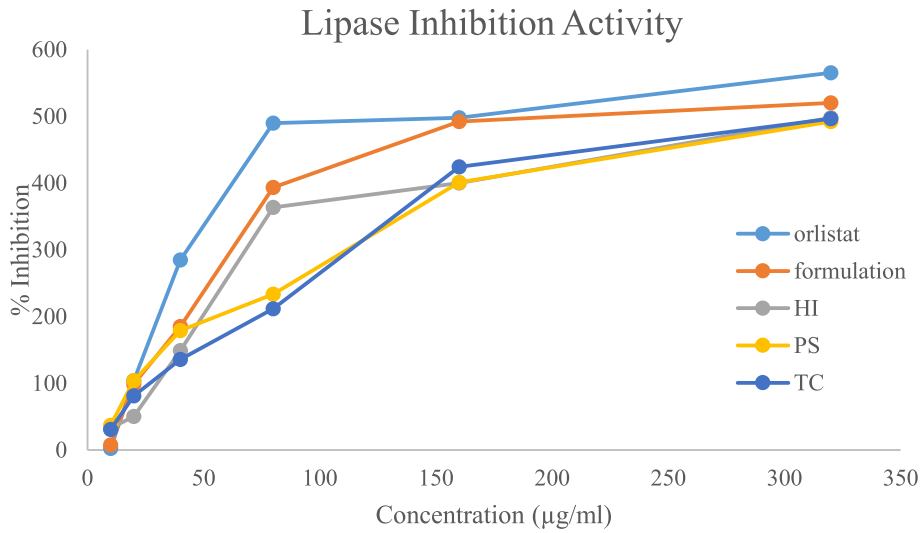

Fig. 1 In vitro lipase inhibition activity data

Results of anti-oxidant activity

Results of anti-oxidant activity are shown in Fig. 2.

Results of formulation

Pre-compression results

Pre-compression results are shown in Table 6.

Post-compression results

Post-compression results are shown in Table 7.

Surface responses of DoE

Surface responses of DoE are shown in Table 8 and Figs. 3, 4, and 5.

The overlay plot (Fig. 6) has two colors which differentiate the runs/trails of formulation into the criteria of the dependent variables. The yellow color shows the region which satisfies the criteria whereas the grey region shows the unsatisfied region. The best results are obtained by the formulation F6 and is considered as the optimized one.

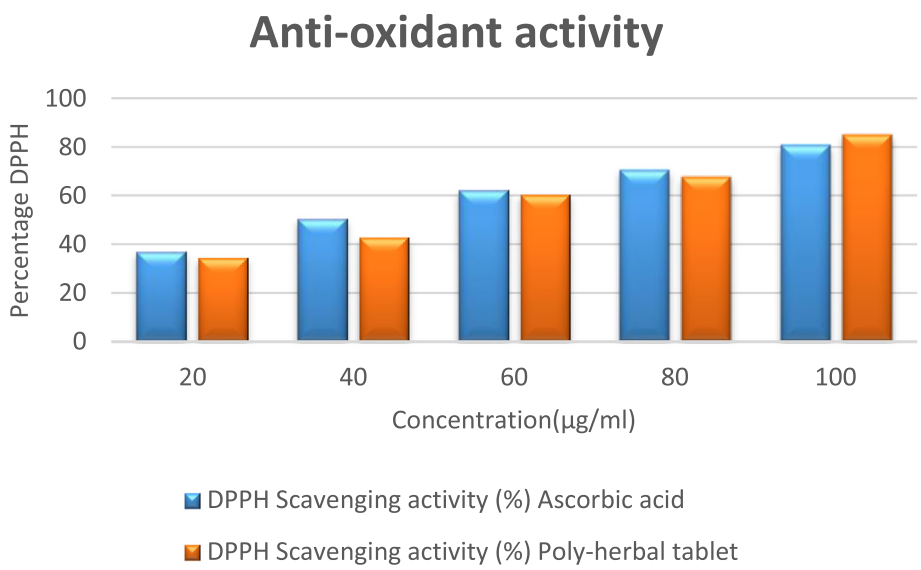

Fig. 2 DPPH scavenging activity data

Table 6 Pre-compression study data

Formulation	Angle of repose (°)	Loose bulk density (g/cm^3)	Tapped bulk density (g/cm^3)	Carr's Compressibility Index	Hausner ratio
F1	32.52 ± 0.5321	0.5712 ± 0.0063	0.6614 ± 0.006107	13.63 ± 0.7156	1.157 ± 0.0095
F2	34.21 ± 0.2264	0.5626 ± 0.0050	0.6724 ± 0.002302	16.32 ± 0.8488	1.195 ± 0.0120
F3	31.13 ± 0.1846	0.5724 ± 0.0252	0.6768 ± 0.027289	15.43 ± 0.7505	1.182 ± 0.0104
F4	32.42 ± 0.3523	0.5774 ± 0.0141	0.6846 ± 0.010502	15.66 ± 0.8256	1.185 ± 0.0116
F5	35.43 ± 0.2906	0.5594 ± 0.0038	0.6522 ± 0.001924	14.22 ± 0.5965	1.165 ± 0.0080
F6	34.67 ± 0.2776	0.567 ± 0.0040	0.6528 ± 0.00249	13.14 ± 0.8698	1.151 ± 0.0114
F7	33.01 ± 0.2197	0.5444 ± 0.0032	0.633 ± 0.002121	13.99 ± 0.4103	1.162 ± 0.0055
F8	32.92 ± 0.406362	0.5518 ± 0.0121	0.6526 ± 0.01506	15.44 ± 0.1561	1.182 ± 0.0021
F9	32.45 ± 0.9388	0.55 ± 0.0131	0.6506 ± 0.011929	14.11 ± 0.6118	1.164 ± 0.0083

Accelerated stability testing

Accelerated stability testing is shown in Table 9.

Discussion

Phytochemical investigations

The pre-formulation studies start with the phytochemical investigations. In the phytochemical investigation, all the three extracts were tested for phytochemical presence. Qualitative screening of phytochemicals is carried out separately for the three extracts. Qualitative screening of major classes like alkaloids, carbohydrates, flavonoids, tannins, and steroids is carried out. The results of these phytochemical tests revealed the presence of alkaloids, tannins, and flavonoids in the extract of Indian elm; presence of alkaloids, tannins, and carbohydrates in the extract of kodo millet; and the presence of tannins in the extract of myrobalan. The results of phytochemical investigations are shown in Table 3.

Physicochemical investigations

The physicochemical investigation is one of the important investigation test in the pre formulation area of the herbs and extracts. The parameters carried out under the physicochemical investigations were loss on drying (LOD), ash values (total ash, acid insoluble ash value, water soluble ash value), and extractive values (alcohol soluble extract, water soluble extract). The extracts are treated separately for the physicochemical investigations. The results are presented in Table 4.The highest percentage of loss on drying (LOD) was shown by Indian elm (28%) followed by myrobalan (23%) and kodo millet (22%). The result of total ash value parameter was shown highest by Indian elm (11%), kodo millet (2.5%), and myrobalan (2.5%). In case of acid insoluble ash value parameter Indian elm (2%), kodo millet (1%), and myrobalan (1%), where as in the case of water insoluble ash value parameter Indian elm, kodo millet, and myrobalan, all showed up the same percentage value that is 1.5%. Extractive value parameter was carried out as alcohol soluble extract and water soluble extract. Indian elm showed the lowest alcohol soluble extractive value (7.5%), kodo millet (10.75%), and myrobalan (17.7%). In water soluble extractive value, kodo millet is the lowest value (13.5%) whereas myrobalan showed 19.5% and Indian elm 37.5%.

Table 7 Post-compression study data

Formulation	Hardness (kg/cm^2)	Thickness (mm)	Diameter (mm)	Friability (%)	Weight variation (%)	Disintegration (min)
F1	2.21 ± 0.0849	3.021 ± 0.011	11.00 ± 0.0082	0.65 ± 0.0230	417.9 ± 3.4777	16.01 ± 0.4070
F2	2.56 ± 0.0334	3.02 ± 0.012	11.00 ± 0.0085	0.56 ± 0.015	420.35 ± 3.8289	15.28 ± 0.2306
F3	2.77 ± 0.0567	3.01 ± 0.013	11.004 ± 0.0069	0.61 ± 0.01	421.5 ± 3.9934	16.39 ± 0.2393
F4	2.04 ± 0.0516	3.01 ± 0.012	11.003 ± 0.0082	0.57 ± 0.005	421.75 ± 2.7120	14.32 ± 0.2884
F5	2.87 ± 0.078	3.02 ± 0.006	11.002 ± 0.0078	0.59 ± 0.005	420.1 ± 3.3701	15.33 ± 0.1981
F6	3.515 ± 0.022	3.02 ± 0.091	11 ± 0.00471	0.52 ± 0.001	421 ± 2.67542	13.05 ± 0.2744
F7	2.28 ± 0.04714	3.02 ± 0.0074	11.006 ± 0.00069	0.62 ± 0.005	420.65 ± 2.1343	14.26 ± 0.2331
F8	2.47±0.0948	3.02 ± 0.0084	11.002 ± 0.0091	0.58 ± 0.004	422.3 ± 3.5108	15.14 ± 0.1615
F9	2.56 ± 0.0948	3.01 ± 0.0103	11.007 ± 0.0082	0.64 ± 0.017	423.1 ± 2.9181	17.27 ± 0.1860

Table 8 Responses

Response	Sum of squares	df	Mean square	*p* value	R^2	Model
Y1	1.07	2	0.5359	0.0263	0.7026	Significant
Y2	0.0109	3	0.0036	0.0336	0.8002	Significant
Y3	10.67	3	3.59	0.0188	0.8429	Significant

In vitro lipase activity

In vitro lipase activity was carried out for all the three extracts separately as well as the formulation and compared with the standard orlistat. Increasing concentration from 10–320 μg/ml were taken to carry out the in vitro lipase activity. Collective results of the in vitro lipase activity are presented in the Table 5 and the graph of the activity in Fig. 1. From concentration level 10, 20, and 40 μg/ml, kodo millet showed up the higher response when the in vitro lipase activity was conducted and the response was 37.01431 (10 μg/ml), 103.8855(20 μg/ml), and 179.1411(40 μg/ml). At level 80 μg/ml (363.39), Indian elm had the highest response when compared to other extracts. At level 160 μg/ml (424.33), myrobalan showed up the highest, where in at level 320 μg/ml, the highest as well as similar response was shown up by both myrobalan (496.314) and Indian elm (497.750). In case of formulations, there was an increase in response with increase in the concentration of the extract mixtures, so the combination of the extract was preferred [9–11].

Anti-oxidant activity

DPPH scavenging activity of the formulation was carried out with ascorbic acid as the reference standard. Concentration range 20–100 μg/ml was selected, and the solutions of both sample and standard used were prepared freshly. The response of the activity showed there was an increase in response with increase in concentration in the selected range. The results are shown in Fig. 2.

Pre-compression parameters

In the formulation of the poly-herbal tablets, the pre-compression parameters performed were angle of repose (θ), loose bulk density (g/cm^3), tapped bulk density (g/cm3), Carr's Compressibility Index, and Hausner ratio. For the better compression of the tablet, it is important that the pre-compression parameters show a better response. The response reveals the flow properties, moisture content, and compressing properties. Data of the pre-compression parameter is presented in Table 6.

Post-compression parameters

Post compression parameters are the quality control aspect of the compressed tablet. Variation in the ratio of binder and disintegrant is responsible for the variation in response of hardness, friability, and disintegrations. The other quality control parameters reported are thickness, diameter, and weight variation. Collective results of post-compression are shown in Table 7.

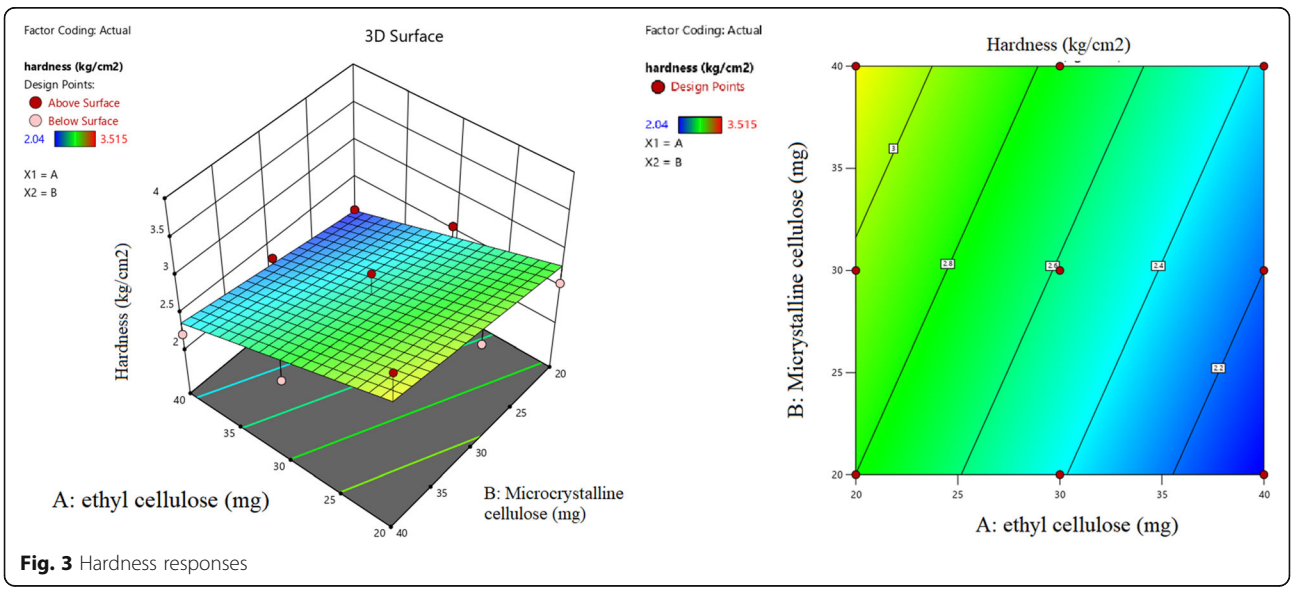

Fig. 3 Hardness responses

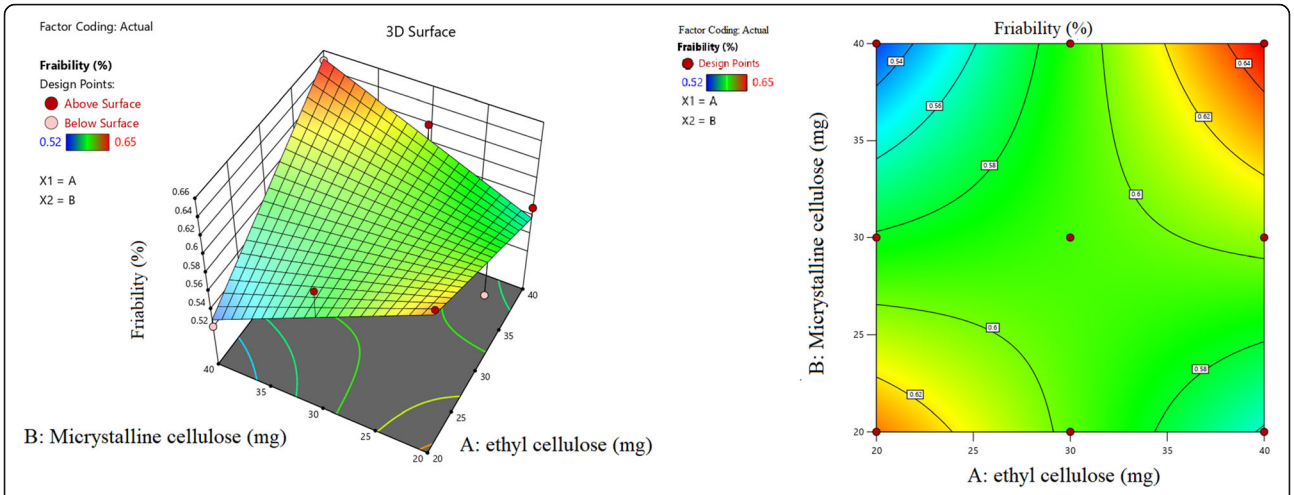

Fig. 4 Friability responses

Design expert (DoE)

The Design Expert 12 software is used in the present study for the formulation design. The 3^2 factorial design is applied where two independent variables are selected, binder (ethyl cellulose) and disintegrant (Microcrystalline cellulose), which have three different levels, low (− 1), intermediate, (0) and high (+1), which are considered. Based on this variables, nine different formulations are designed with varying ratios of the binder and disintegrant. The results obtained by conducting evaluation parameters of tablets are added to the software DoE for obtaining the results for model significance. The responses are recorded in the form of hardness, friability, and disintegrant for all the nine formulations .The response data is shown in the Table 8 and response surfaces are shown for hardness (Fig. 3), friability (Fig. 4), and disintegration time (Fig. 5). Increase in binder will increase the hardness and decrease in the friability and disintegration time will increase. Increase in the concentration of disintegrant will reduce the disintegration time and decrease the hardness level. Hence, the optimum ratio of the binder and disintegrant is require. The formulation F6 shows the optimum ratio. The DoE surface response of all Y1, Y2, and Y3 showed that the model was significant as the p value was less than 0.05% and the overlay plot of the trails shows that formulation F6 satisfies all the criteria better among all the nine formulations and is further considered for optimization [12–14].

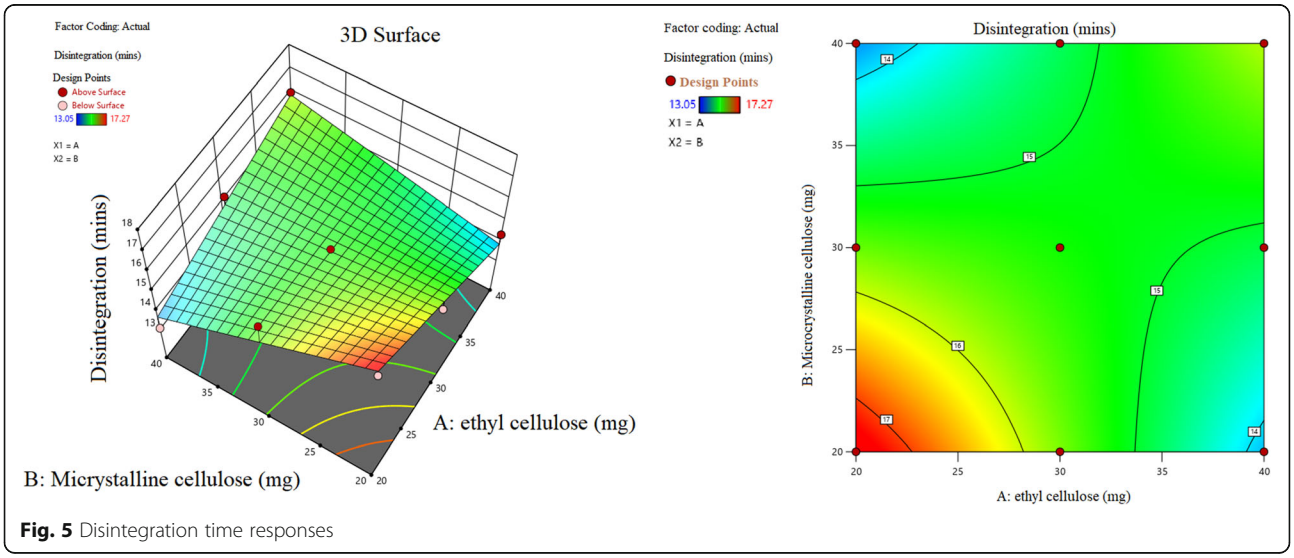

Fig. 5 Disintegration time responses

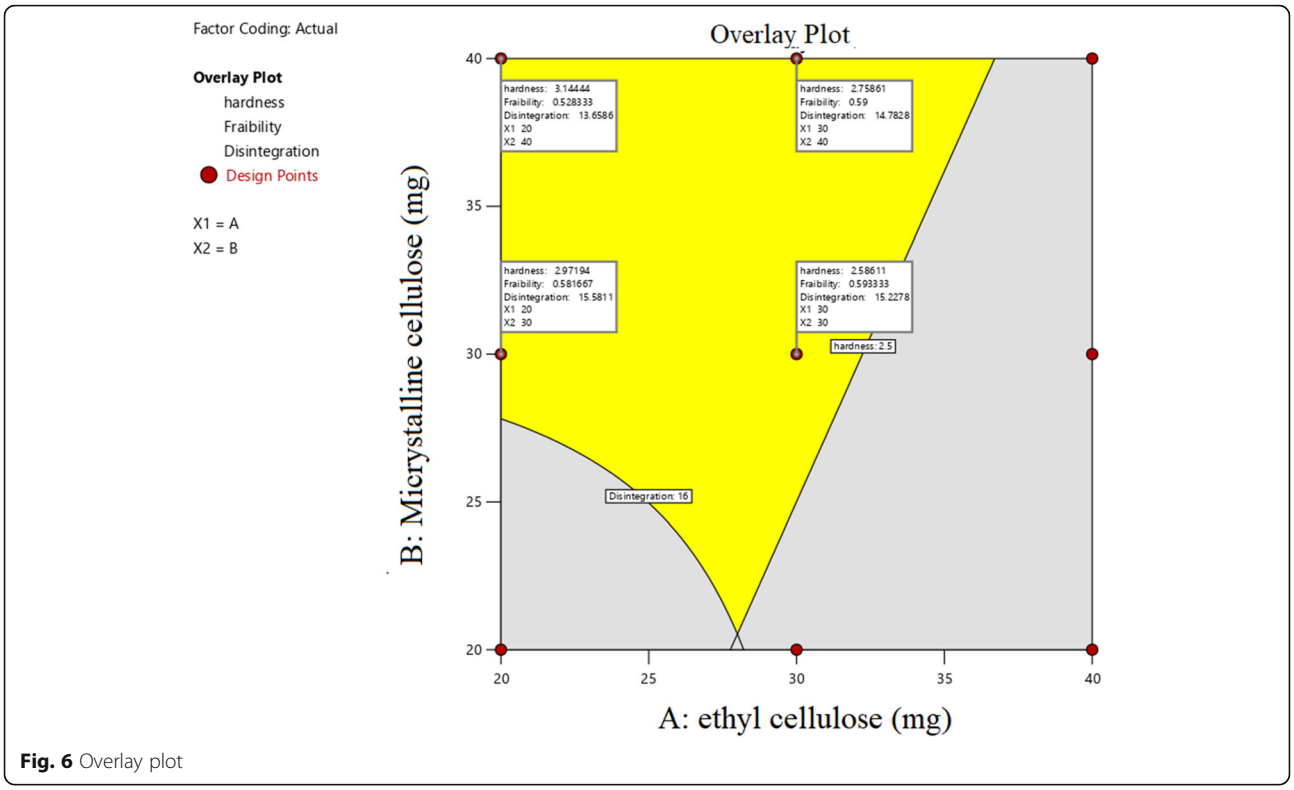

Fig. 6 Overlay plot

Accelerated stability testing

The stability study was conducted for formulation F6 for duration of 30 days at two different conditions, one at room temperature (25 °C ± 2 °C/RH 60 ± 5%) and accelerated temperature (40 °C ± 2 °C/RH 75 ± 5%), and the results (Table 9) showed that the poly-herbal tablets were stable [15].

Conclusion

The phyto-chemical investigation and the phyisco-chemical were carried out prior to the poly-herbal tablet formulation. The in vitro anti-lipase activity was carried out for a different concentration . The 3^2 factorial design was applied by using the design of experiment software, and the 9 different formulations were prepared with variation in disintegrating agent and binding agent ratios. The formulation F6 with (20:40 ratio) showed better results of the evaluation parameters conducted. The formulation F6 was considered the better one and stability studies were conducted with showed good stability results. Although the extracts showed up to have the inhibitory activity against the pancreatic enzymes in the in vitro study, but it cannot be considered effective on human beings until the pre-clinical and clinical studies are carried out which are required to prove the efficiency of the extracts. Results of the in vitro study can be considered as the background highlights for the future investigations of the herbal extracts which can be developed to the medicinal value ingredient for treatment and prevention of obesity and related metabolic diseases.

Table 9 Accelerated stability study data

Parameters	Initial	Room temperature			Accelerated temperature		
		25 °C ± 2 °C/RH 60 ± 5%			40 °C ± 2 °C/RH 75 ± 5%		
		7th day	15th day	30th day	7th day	15th day	30th day
Hardness (kg/cm^2)	3.51	3.51	3.55	3.53	3.50	3.49	3.48
Friability (%)	0.52	0.52	0.51	0.52	0.52	0.53	0.51
Disintegration (min)	13.05	13.05	13.06	13.06	13.10	13.16	13.09

Abbreviations
WHO: World Health Organization; PL: Pancreatic lipase; LOD: Loss on drying; DMSO: Dimethylsulphoxide; PNPB: 4-Nitrophenyl butyrate; DPPH: (2, 2-diphenyl-1-picrylhydrazyl); PEG: Polyethylene glycol; DoE: Design of experiments

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Authors' contributions
AB has done designed the formulations and carried out the formulation procedures of poly-herbal tablet as well as the pre-compression and post-compression studies along with the stability studies. AS has carried out the collection, extraction, and the in vitro activity. MP has designed the concept, corrections, and drafting of the manuscript. The authors have read and approved the manuscript.

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