*Research Article*

# Development of poly-herbal tablets of *Inula racemosa* and *Ceratonia siliqua* plant extracts by design expert tools

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**Abstract**

**Background:** Obesity is a lifestyle related disorder that is rapidly increasing day by day due to fast-paced life. Many treatments available in the market claim to treat obesity, but comes with masses of side effects. That's why people are getting attracted by Ayurveda and Ayurvedic medicinal approaches for the treatment of obesity. **Objective:** The current study aimed to formulate herbal tablets of *Inula racemosa* and *Ceratonia siliqua* plant extracts by performing all the possible pre-formulation studies required in the development process. **Material and methods**: Various pre-formulation studies like organoleptic properties, bulk characterization, physiochemical investigation, phyto-chemical investigations etc were performed. Excipient compatibility study in solid state in ratio a 1:1 was also performed prior to formulation development. **Results and conclusion**: A regular 2-level factorial design (2FI) with three center points was applied for the formulation optimization design of poly-herbal tablet. The stability study for poly herbal tablets of *Inula racemosa* and *Ceratonia siliqua* were performed and the formulation found stable after three months at accelerated conditions. Formulation F5 showed best result among all other formulations that were reproduced as formulation F6 and F7. The performed stability study also showed that the formulation is stable at 40°C temperature and 75%RH for 3 months. The whole study gives a brief idea and a support structure for developing *Inula racemosa* and *Ceratonia siliqua* plant extract tablets.

***Keywords*:** *Inula racemosa*, *Ceratonia siliqua*, Pre-formulation Studies, DOE, Stability studies

## Introduction

Obesity is a life-style related disorder that never comes alone but it takes a lot of related disease with itself. With increasing inactive lifestyle, weight management now a day's is a very difficult and challenging task. With changing lifestyle there is decline in cereal intake and increase in sugar and fat that leads to deposition of fat inside human body. And once it get deposited, then fatty substances make adipose tissue a home for itself. For getting rid of increased weight people try a lot of supplements, therapies and drug regimes. But few treatments come with a lot of adverse effect and few leads to rebound weight gain (Hsu and Yen, 2007).

From ancient times plants are always an exemplary and extra-ordinary source of drugs and there are a lot of proven herbal products available in the market that claims to treat obesity. *Inula recemosa* also called pushkarmool is a mentioned and proven herb for weight loss in "Charak Samhita", where as *Ceratonia siliqua* also known as locust bean is known for lowering lipid level of blood in western countries (Chirmadel et al., 2015; Flora of China, 1881; Kumazawa et al., 2022). The activities of both the drugs alone or in combination were recently studied and the anti-obesity activity is reported in albino mice on gold thioglucose induced adiposity. These herbs contain various bioactive constituents that have been known for weight management (Singha et al., 2024).

Following recent trends, nowadays people prefer the ayurvedic approach over the allopathic approach for minimizing side effects as chemically synthesized drugs have many side effects on the human body. Herbal products may contain a single herb or a combination of several different herbs believed to have complementary and

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synergistic effects with very less side effects on the human body (Singha et al., 2024; Balekundri et al., 2020).

The administration of drugs by oral route is considered as most convenient route for drug administration. Our current study aimed at formulating tablets of *Inula recemosa* and *Ceratonia siliqua* for weight management. This study is not only based upon the formulation and evaluation of tablets made from *Inula racemosa* and *Ceratonia siliqua* plant extracts but also covers all the aspects involved in formulating herbal tablets. The concept of QBD (i.e. quality by design) was implemented to understand all the elements involved in formulation and development process. This paper also gives a brief idea about the implementation of design of expert (DOE) in developing herbal medicines. All the experiment protocols were designed as per QBD and the dose of drug content is defined as per the study performed for both the drugs on gold thioglucose induced obesity (Singha et al., 2024).

**Material and methods**

**Pre-formulation study**

Pre-formulation study prior to compounding process of formulation was studied by studying physical and chemical properties of drug components. In pre-formulation study of *Inula racemosa* and *Ceratonia siliqua* plant extract, the nature and various characteristics of each and every component was studied in order to optimize conditions of the drug component for final formulation.

**Organoleptic Characters**

*Inula racemosa* and *Ceratonia siliqua* plant extracts were evaluated for colour, Taste and Odor by physical method of examination.

**Bulk Characterization**

*Inula racemosa* and *Ceratonia siliqua* plant extracts were investigated for Particle size characterization, Rheological Properties (Powder flow) and Moisture Uptake Study.

**Particle Size Characterization**

Particle Size Characterization of *Inula racemosa* and *Ceratonia siliqua* plant extracts were carried out by using sieve test method with the help of mechanical sieve shaker apparatus. Plant extract of *Inula racemosa* and *Ceratonia siliqua* plant extracts was taken and dried in hot air oven for 10-15 minutes. A known amount of plant extract was taken and weighed accurately (Sample must not exceed 500 grams). Stack of sieves were prepared in ascending order (i.e. lower sieve number is placed at the top and higher is placed at the bottom). A pan is attached under the lowest sieve in order to collect retained material of highest sieve. The nest is fitted to the mechanical sieve shaker. The whole nest was shaking for 10 minutes horizontally at 280 oscillations per minute. Powder from each sieve was collected and % retained from each sieve was calculated.

**Rheological Properties**

**Bulk Density (BD):** The bulk density of the powdered extract is measured by filling known amount of powder in graduated measuring cylinder. The volume of graduated cylinder is measured and bulk density of powder is calculated by using formula:

*Bulk Density: Mass of powder/Volume of powder in measuring cylinder*

**Tapped Density (TD):** The tap density apparatus was used for determining tapped density of powders. The known amount of powder was filled in graduated cylinder of tap density apparatus and then the graduated cylinder was fixed to apparatus. The testing apparatus was tapped for 100 times at 14 mm of height. After 100 taps the volume of cylinder was measured and tapped density is calculated by using:

*Tapped Density: Mass of powder/Final Volume of powder in measuring cylinder*

**Moisture Uptake Study:** Moisture uptake study was done by using conventional Petri-plate method. A known amount of powder was weighed and kept in Open Petri-plate in open condition. The weight gain from initial state was studied for 15 days at normal room temperature and pressure. Then moisture uptake capacity was calculated as:

*MUC = (Initial Weight – Final Weight)/Initial Weight x 100*

**Physiochemical Investigation**

**Total Ash Value:** Weigh about 2–5 g of plant extract and then take it into the crucible, keep it at a temperature of about 500 °C–600 °C in the muffle furnace till the plant extract forms carbon-free ash, then it is cooled, weigh the ash formed (Balekundri et al., 2020). Calculate Total Ash Content (%) using the formula:

*Total Ash (%) = (Weight of total ash obtained/ Weight of crude drug) x 100*

**Acid Insoluble Ash:** Obtained Carbon free ash is boiled with exact 25 ml of Hydrochloric Acid for 5 minutes. Collect insoluble matter in crucibles (sintered) with ash less filter paper and then the sample with hot water and ignite it at 500°C till a constant weight is observed. Then calculate acid insoluble ash content by using following formula.

*%Acid Insoluble Ash = (Weight of acid Insoluble Ash/Weight of the crude drug) x 100*

**Water Soluble Ash:** Obtained Carbon free ash is boiled with 25 ml of purified water; it was then collected in sintered crucibles with the help of ash less paper, was the sample with hot Purified water and ignited at 500°C to obtain constant weight.

*%Water Soluble Ash= (Wt of total Ash – Wt. of Water Insoluble ash)/ Wt. of Crude drug x 100*

**Extractive Values**

**Alcohol Soluble Extractives:** To 4 grams of plant extract sample add 25 ml of alcohol in flask and kept it for 24 hours. Filter the alcoholic solution through regular filter paper (8 um). Pour the filtrate in Petri-plates and kept at 105°C temperature for 5-6 hours and weight accurately the final extract.

*% Alcohol Soluble Extractive = (Weight of Extract/Weight of Plant Material) x 100*

**Water Soluble Extractives:** To 4 g of plant extract sample add 25 ml of Purified Water in a volumetric flask. The flask was kept aside for 24 hour. Pour the filter in petriplates and kept at 105°C temperature for 5-6 hours and weight accurately the final extract.

*%Water soluble Extractive = (Weight of Extract/Weight of Plant material) x 100*

**Loss on Drying (LOD):** The LOD of plant extract sample(s) were measured by using IR moisture analyzer with 1 gram of sample at 105°C. Observation displayed on HMI was noted as final result.

**Qualitative Phytochemical Investigations of Plant Extracts**

The phyto-chemical investigation was carried out for all crude drug materials for the identification of different classes. Phyto-chemical screening of plant extracts was done by standard phyto-chemical screening methods. Different phyto-chemical constituents like alkaloids, amino acids, carbohydrate, volatile oil, flavonoids, glycosides, tannins, terpenoids and steroids were detected in the plant extract by qualitative using standard tests (Trease and Evans, 2017; Sahira et al., 2015; Morsy et al., 2014; Gupta et al., 2013).

**Quantitative Estimation of Bioactive Constituents**

**Determination of Total Flavonoid Content**: Total flavonoid content of plant extract was evaluated with Jiao method by using Quercetin as reference standard. 1 ml of plant extract or standard was taken at different concentrations in test tube. 200 µl of 10% aluminum chloride solution was then added, after that 200 µl of 1 M potassium acetate was added in the same test tube. 5.6 ml of distilled water was finally added to the reaction mixture. Reaction mixture was then incubated for 30 minutes at room temperature in order to complete the reaction. The absorbance of standard and test was measured at 415 nm wavelength using UV Spectrophotometer (Rebaya et al., 2014; Rahman et al., 2012). Total flavonoid content was calculated by following equation:

$$Total\ Flavonoid\ content\ (TPC) = C \times V / M$$

Where: C = QE Concentration, V = Volume of drug used, M = Mass of drug

**Determination of Total Phenolic Content:** Total phenolic content of the plant extract was evaluated with the help of Folin-Ciocalteu method by using gallic acid as reference standard. 0.5 ml aliquots of different concentration were reacted with 2.5 ml 0.2 mol/l folin ciocalteu reagent (for 4 minutes). Then 2ml of saturated sodium carbonate solution (about 75g/l) was added to reaction mixture. The reaction mixture was incubated for 2 hours after that the absorbance reading was taken at 760 nm (Uddin et al., 2015). Total phenolic content were calculated by following equation:

$$Total\ Phenolic\ content\ (TPC) = C \times V / M$$

Where; C = Concentration of gallic acid, V = Volume of drug used, M = Mass of drug

**Determination of Total Tannin Content:** The tannin content or proanthocyanidin were determined by method of Broadhurst et al. (1978) with slight modifications by using tannic acid as reference standard. Different concentration (10, 50, 100, 250, 500 µg/Ml) of plant extract was added to different test tubes. After that 3 mL of a vanillin solution (in 4% methanol) and 1.5 ml of concentrated hydrochloric acid was added to test tubes. After incubation for 15 minutes, the absorbance was measured at 500 nm. Total tannin content was calculated by following equation:

$$Total\ Tannin\ content\ (TTC) = C \times V / M$$

Where; C = Concentration of tannic acid, V = Volume of drug used, M = Mass of drug

**Determination of Total Alkaloid Content:** Total Alkaloid content of the plant extract was evaluated with the help of Bromocresol method by using Atropine as reference standard. 1 mg of plant extract was dissolved in dimethyl sulphoxide (DMSO) was added in test tubes. The reaction mixture was then added with 1ml of 2N Hydrochloric Acid and filtered. The solution was then transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer was added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. Absorbance was measured at 470 nm using spectrophotometer (Tambe et al., 2014). Total

Alkaloid content was calculated by following equation:

$$Total\ Alkaloidal\ content\ (TAC) = C \times V / M$$

Where; C = Concentration of tannic acid, V = Volume of drug used, M = Mass of drug

**Separation of Bioactive Constituents**

**Thin Layer Chromatography method:** Standard analytical procedure was used for the separation of various bioactive constituents of plant extracts (Stahl et al., 1958). Before starting the study the plant extract of *Inula racemosa* and *Ceratonia siliqua* was solublized in methanol referring as methanolic extract of *Inula racemosa* (MEIR) and methanolic extract of *Ceratonia siliqua* (MECS). Along with that suitable solvent systems were prepared as per standard procedures.

**Confirmation of Bioactive Constituents by HPLC**

**Analytical Testing for Assay:** Assay content determination help in quantifying certain components of plant extract that not only give idea about potency of extract used, but also act as analytical markers for product development. In present study *Inula racemosa* and *Ceratonia siliqua* plant extracts were separately analyzed for alantolactone and proanthocyanidins content.

**Assay of *Inula racemosa* Plant Extract**: Assay of *Inula racemosa* plant extract was determined by high performance liquid chromatography (HPLC). Test solution was prepared by extraction process. 2.0 grams of plant extract was refluxed in 200 ml of Methanol for 4 hours in soxhlet apparatus. The extract turns colorless, then it was cooled and filtered. Concentrate under vacuum to 10 ml.

**Chromatographic system:-**Column: 30 cm x 4.6 mm stainless steel column packed with octadecylsilane bonded to porus silica (10µm), Mobile Phase: a mixture of 70 Volumes acetonitrile and 30 Volume Water, Flow Rate: 1ml per minute, Spectrophotometer: Set a 210 nm, Injection Volume: 20 µl, Flow rate: 1.0 ml/min. Calculate the content of alantolactone (Indian Pharmacopeia 2018)**.** The content of alantolactone should not less than 15%.

**Assay of *Ceratonia siliqua* Plant Extract**: Assay of *Ceratonia siliqua* plant extract was determined by high performance liquid chromatography (HPLC). Test solution was prepared by mixing 2.5 grams of plant extract in 30 ml Methanol, mixture was then extracted for 10 minutes in water bath. The solution was cooled to room temperature and the volume was increased up to 100 ml with methanol. The content was filtered by using filter paper.

**Chromatographic system:** Column: C18, Mobile Phase: a mixture of Water: Methanol: Isopropanol: Formic Acid in ratio 73:13:6:8, Spectrophotometer: Set a 525 nm, Injection Volume: 10 µl, Flow rate: 1.0 ml/min (Gray et al., 1999). Calculate the content of proanthocyanidin. The content of proanthocyanidin should not less than 15%.

**Excipient compatibility study**

Excipient compatibility plays important role in understanding role of excipients in product quality. Its selection should be based upon understating of drug substance and their impurities. The excipients used in *Inula racemosa* and *Ceratonia siliqua* tablets were selected based

**Tablet 1: Excipient compatibility (binary mixtures) Scheme**

| S. No | Vial No. | Name of Ingredients | Concentration Ratio |
|---|---|---|---|
| **1** | 1A, 1B, 1C | *Inula racemosa* | 1:1 |
| **2** | 2A, 2B, 2C | *Ceratonia siliqua* | 1:1 |
| **3** | 3A, 3B, 3C | *Inula racemosa* + Microcrystalline Cellulose 200 | 1:1 |
| **4** | 4A, 4B, 4C | *Inula racemosa* + Crospovidone XL-10 | 1:1 |
| **5** | 5A, 5B, 5C | *Inula racemosa* + HPMC E-15 | 1:1 |
| **6** | 6A, 6B, 6C | *Inula racemosa* + Purified Talcum | 1:1 |
| **7** | 7A, 7B, 7C | *Inula racemosa* + Colloidal Silicon Dioxide | 1:1 |
| **8** | 8A, 8B, 8C | *Inula racemosa* + Magnesium Stearate | 1:1 |
| **9** | 9A, 9B, 9C | *Ceratonia siliqua* + Microcrystalline Cellulose 200 | 1:1 |
| **10** | 10A, 10B, 10 C | *Ceratonia siliqua* + Crospovidone XL-10 | 1:1 |
| **11** | 11A, 11B, 11C | *Ceratonia siliqua* + HPMC E-15 | 1:1 |
| **12** | 12A, 12,B,12C | *Ceratonia siliqua* + Purified Talcum | 1:1 |
| **13** | 13A, 13B, 13C | *Ceratonia siliqua* + Colloidal Silicon Dioxide | 1:1 |
| **14** | 14A, 14B, 14C | *Ceratonia siliqua* + Magnesium Stearate | 1:1 |

Vial numbered as A: Represents Vials charged at controlled room temperature, Vials B represents Open condition at 40 °C/75 % RH and Vials C represents Closed conditions

on excipient compatibility study. Drug- Excipient study was assessed through physical examination (i.e. Change in color and Odor) of binary mixture of excipient with drug at ratio 1:1 in solid state. Sample was stored at 40°C/75 % RH in both open and closed glass vials for 1 month. Common excipient functioning as diluents, disintegrants, glidants and lubricants and tablet 1 is representing the excipient compatibility scheme.

**Process selection**

Both the powders are having BD=0.65g/ml (for *Inula racemosa*) and 0.70 g/ml for *Ceratonia siliqua* respectively that indicate the powder is exhibiting behavior like sand. Thus initially direct compression of blend was performed; direct compression was considered as acceptable approach for this formulation. Wet granulation method was also not opted as the plant extracts contains various phenolic contents that may degrade upon drying.

**Formulation development**

A regular 2 level factorial design (2FI) with three center points was applied for the formulation optimization design of poly-herbal tablet. The two formulation dependent factors were evaluated at three different response levels. The software Design Expert 13.0.12.0 was used for designing the formulation. The total number of runs is 7 in which 4 runs are having factorial space type and 3 runs are having center space type. All variables were studied by using surface response. The trial scheme is mentioned in table 2 and 3.

**Procedure:**

All the ingredients described in tablet 3 were weight accurately as per Trial Scheme by using calibrated weighing balance. Sieve integrity was checked before starting the sifting operation. *Inula racemosa*, *Ceratonia Siliqua* along with microcrystalline Cellulose 200 was sifted through sieve # 20 and Crospovidone XL-10, HPMC E-15, Purified talcum and colloidal silicon dioxide was sifted through sieve #40 and was collected in double lined polybag. Magnesium Sterate was sifted separately through seive #60 and was collected in double lined polybag. All ingredients were dried at 60°C temperature for complete 1 hour in order to remove excess moisture. All the sifted ingredients were loaded in double cone blender except Magnesium Stearate and was blend for 30 minutes. After that add magnesium stearate was added to it and again blend for 05 minutes. The blend is then ready for compression. The tablets were compressed with the help of compression machine and all the physical parameters were noted.

***Punch Tooling:*** *16.75X7.90 mm, Capsule shaped, Standard Concave Plain on Both sides.*

Perform the same aforementioned procedure for Trial F1 to F7.

**Formulation Parameters for Pre-Compression Studies**

**Rheological Properties:** Rheological properties like Bulk density, Tapped density, Carr's Index and Hausner ratio was performed for the blend ready of compression. Procedure for Bulk density and Tapped density remained same as per pre-formulation study parameters.

**Carr's Index:** The compressibility index of powder is calculated by using following formula:

*Carr's Index = (BD-TD)/BD X 100*

**Hausner Ratio:** Hausner ratio is calculated by using following formula.

*Hausner ratio = BD/TD*

**Loss on Drying (LOD):** The LOD of final blend was measured by using IR moisture analyzer with 1 gram of sample at 105°C. Observation displayed on HMI was noted as final result.

**Formulation Parameters for Post Compression Studies**

**Average Weight:** The weight of the compressed tablet was measured by using calibrated weighing balance (Essae

**Table 2: Representation of Factor-Response scheme as per Design of Experiment**

| Run | Block | Group | Build Type | Space Type | Row Status | Factor 1 A:Crospovidone XL-10 | Factor 2 B. HPMC E15 | Response1 Hardness | Response 2 Friability | Response 3 Disintegration Time |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Block 1 | 1 | NA | Factorial | Norm al | 14.000 | 12.000 | - | - | - |
| 2 | Block 1 | 1 | NA | Factorial | Normal | 18.000 | 8.000 | - | - | - |
| 3 | Block 1 | 1 | NA | Factorial | Normal | 16.000 | 16.000 | - | - | - |
| 4 | Block 1 | 1 | NA | Factorial | Normal | 10.000 | 10.000 | - | - | - |
| 5 | Block 1 | 1 | NA | Center | Normal | 16.000 | 10.000 | - | - | - |
| 6 | Block 1 | 1 | NA | Center | Normal | 16.000 | 10.000 | - | - | - |
| 7 | Block 1 | 1 | NA | Center | Normal | 16.000 | 10.000 | - | - | - |

**Table 3: Complete trial scheme of different formulations**

| S. No | Name of ingredient | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|---|---|
| **1** | *Inula racemosa* | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 |
| **2** | *Ceratonia siliqua* | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 | 200.000 |
| **3** | Microcrystalline Cellulose 200 | 196.000 | 196.000 | 184.000 | 196.000 | 190.000 | 190.000 | 190.000 |
| **4** | **Crospovidone XL-10** | **14.000** | **18.000** | **16.000** | **10.000** | **16.000** | **16.000** | **16.000** |
| **5** | **HPMC E-15** | **12.000** | **8.000** | **16.000** | **10.000** | **10.000** | **10.000** | **10.000** |
| **6** | Purified Talcum | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 |
| **7** | Colloidal Silicon Dioxide | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **8** | Magnesium Stearate | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 |
| **Average Weight of Core Tablet** | | **630.000** | **630.000** | **630.000** | **630.000** | **630.000** | **630.000** | **630.000** |

Teraoka PVT. Ltd- Max Capacity: 220 grams) and was recorded in mg unit.

**Weight Variation:** The weight variation of the compressed tablet was measured by using calibrated weighing balance (Essae Teraoka PVT. Ltd- Max Capacity: 220 grams) and was recorded in mg unit. 20 tablets from each batch was individually weight, the average weight of tablet was calculated and then further calculates as:

*Weight Variation = Individual Weight of the tablet/ Average Weight of tablets x 100*

**Diameter:** The diameter of the tablets was measured by using vernier caliper (INSIZE - Code no.: 1112-150) and the diameter is recorded in mm. (Note tablet diameter should within Standard tablet diameter ± 0.2 mm).

**Thickness:** The thickness of the tablets was measured by using vernier caliper (INSIZE - Code no.: 1112-150) and the thickness is recorded in mm. (Note tablet thickness should within Standard tablet thickness ± 0.2 mm).

**Hardness:** The hardness of tablets was measured by using tablet hardness tester (Electrolab– EH01) and is recorded in kgf. (Note hardness of tablets should not less than 3 Kgf).

**Friability:** Friability of tablets was measured by using friability test apparatus (Elecrolab EF2). Ten tablets from each batch were taken and initial weight of 10 tablets was noted. These tablets were then placed in friability test apparatus and are rotated for elapsed time of 4 minutes at 25 RPM (i.e. 100 Rotations). Finally the tablets were removed and de-dusted with brush. Then final weight of tablets was recorded as final weight. The % friability of tablets was calculated. (Note: The friability of tablet should not more than 1%).

*% Friability = (Initial Weight – Final Weight)/ Initial Weight x 100*

**Disintegration Time:** The disintegration apparatus of tablet were carried out by using Disintegration Test Apparatus, 900 ml of purified water was added to disintegration test vessel and 6 tablets of each batch was placed in each tube. The temperature is maintained at 37°C±2°C. The time tablet to disintegrate the tablets was recorded. (Note: The disintegration time of tablets should not more than 15 minutes for uncoated tablets).

**Analytical Testing:** All the samples were analyzed as per described assay content determination method.

**Stability studies**

The final optimized formulations i.e. F5, F6 & F7 were packed in HDPE bottles with Child resistant caps and the accelerated stability study of *Inula racemosa* and *Ceratonia siliqua* herbal tablets were done at $40^0$C temperature & 75% Relative humidity for 3 months. The entire samples were analyzed for assay content at the completion of 3 months.

**Results**

**Pre-formulation Study**

**Organoleptic Characters:** The observed organoleptic characters of *Inula racemosa* and *Ceratonia siliqua* plant extracts are described in Tablet 4 and Figure 1.

**Bulk Characterization**

**Particle Size Characterization:** Particle size may interfere physical as well as chemical properties of plant extract and is an important indicator or product quality. From the results it was observed that maximum percentage passing is through sieve #10 i.e. 2000 µm that decreases with decrease in sieve apertures size for both *Inula racemosa* and *Ceratonia siliqua* plant extract. Results of particle size characterization by using sieve analysis method are described in Table 5 and 6.


**Figure 1:** Visual representation of *Inula racemosa* and *Ceratonia siliqua* plant extract.

**Rheological Properties:** The Rheological properties govern the flow behavior of plant extracts of *Inula racemosa* and *Ceratonia siliqua*. Bulk density of loose powder indicates the powder is having properties just like fine to medium sand. Tap Density of plant extract indicates that the powder is trappable and powder is suitable for direct compression. Results are tabulated in Tablet 7.

**Moisture Uptake Study:** Moisture uptake study is crucial parameters for indicating moisture absorption on product stability. *Inula racemosa* and *Ceratonia siliqua* plant extract absorb water upto 4.678 grams (for *Inula racemosa*) and 3.656 grams (for *Ceratonia siliqua*) from initial weight indicate

**Table 4:** Representation of Organoleptic Characteristics of *Inula racemosa* and *Ceratonia siliqua* extracts

| Particulars | *Inula racemosa* Extract | *Ceratonia siliqua* Extract |
|---|---|---|
| Color | Dull Brownish Color | Dark Brown Color |
| Odor | Camphoraceous Odor | Slightly Pungent |
| Taste | Bitter | Sweet |

**Tablet 5:** Particle size Characterization of *Inula racemosa* extract by using sieve analysis method

| Sieve Number (#) | Aperture Size (µm) | Mass of Powder Retained (g) | Individual Percentage Retained (%) | Cumulative mass retained(g) | Cumulative percentage retained (%) | Percentage Passing (%) | Reported Percentage Passing (%) | Fineness Modulus |
|---|---|---|---|---|---|---|---|---|
| **10** | 2000 | 0.000 | 0.000 | 0.000 | 0.000 | 100.000 | 100 | 4.900 |
| **20** | 841 | 12.249 | 12.249 | 12.249 | 12.249 | 87.751 | 88 | |
| **30** | 595 | 20.365 | 20.365 | 32.614 | 32.614 | 67.386 | 67 | |
| **40** | 420 | 39.324 | 39.324 | 71.938 | 71.938 | 28.062 | 28 | |
| **60** | 250 | 12.096 | 12.096 | 84.034 | 84.034 | 15.966 | 16 | |
| **80** | 177 | 8.259 | 8.259 | 92.293 | 92.293 | 7.707 | 8 | |
| **100** | 149 | 4.586 | 4.586 | 96.879 | 96.879 | 3.121 | 3 | |
| **Pan** | NA | 3.127 | 3.127 | 100.006 | 100.006 | 0.000 | 0 | |

**Tablet 6:** Particle size Characterization of *Ceratonia siliqua* Extract by using sieve analysis method

| Sieve Number (#) | Aperture Size (um) | Mass of Powder Retained (g) | Individual Percentage Retained (%) | Cumulative mass retained (g) | Cumulative percentage retained (%) | Percentage Passing (%) | Reported Percentage Passing (%) | Fineness Modulus |
|---|---|---|---|---|---|---|---|---|
| 10 | 2000 | 0.000 | 0.000 | 0.000 | 0.000 | 100.000 | 100 | 4.946 |
| 20 | 841 | 12.249 | 12.249 | 12.249 | 12.249 | 87.751 | 88 | |
| 30 | 595 | 27.893 | 27.893 | 40.142 | 40.142 | 59.858 | 60 | |
| 40 | 420 | 32.988 | 32.988 | 73.130 | 73.130 | 26.870 | 27 | |
| 60 | 250 | 9.876 | 9.876 | 83.006 | 83.006 | 16.994 | 17 | |
| 80 | 177 | 5.987 | 5.987 | 88.993 | 88.993 | 11.007 | 11 | |
| 100 | 149 | 8.098 | 8.098 | 97.091 | 97.091 | 2.909 | 3 | |
| Pan | NA | 2.909 | 2.909 | 100.000 | 100.000 | 0.000 | 0 | |

**Tablet 7:** Representation of rheological properties of *Inula racemosa* and *Ceratonia siliqua* extracts

| Particulars | *Inula racemosa* Extract | *Ceratonia siliqua* Extract |
|---|---|---|
| Bulk Density | 0.65 g/ml | 0.70 g/ml |
| Tapped Density | 0.74 g/ml | 0.82 g/ml |

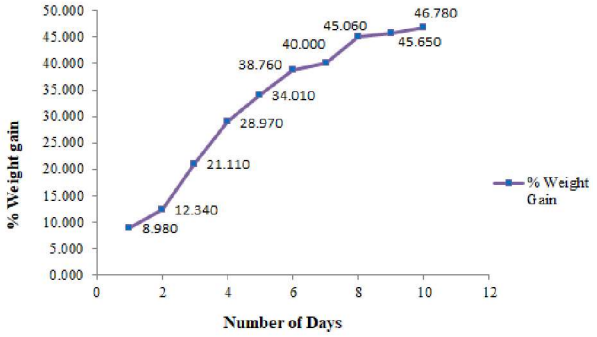


**Figure 2:** Graphical repersentation of moisture uptake study of *Inula racemosa* extract

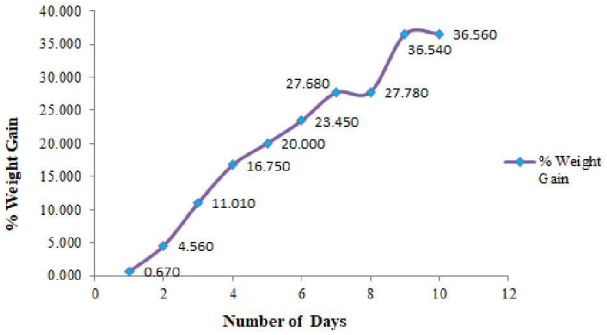


**Figure 3:** Graphical repersentation of moisture uptake study of *Ceratonia siliqua* extract.

hygroscopic tendencies of plant extract. The results of moisture uptake study are tabulated in Figure 2 and 3.

**Phytochemical Investigation of different Extracts**

**Qualitative Analysis:** Preliminary phytochemical screening of the *Inula racemosa and Ceratonia siliqua* extracts showed

**Tablet 8:** Results of phytochemical qualitative analysis of methanolic extract (MEIR) of *Inula racemosa* and methanolic extract of *Ceratonia Siliqua* (MECS)

| S. No. | Phytoconstituents | Tests | | Plant extracts | |
|---|---|---|---|---|---|
| | | | | MEIR | MECS |
| 1. | Acidic compound | a. | Sodium bicarbonate test | + | + |
| | | b. | Litmus paper test | + | + |
| 2. | Alkaloids | a. | Dragendorff test | +++ | - |
| | | b. | Mayer test | + | + |
| | | c. | Wagner test | + | + |
| | | d. | Hager test | + | ++ |
| 3. | Flavonoids | a. | Shinoda test | +++ | + |
| | | b. | Alkaline reagent test | ++ | ++ |
| | | c. | Zinc hydrochloride test | ++ | + |
| 4. | Saponin Glycosides | a. | Froth formation test | ++ | ++ |
| | | b. | Heamolysis test | +++ | +++ |
| 5. | Other Glycosides | a. | Borntrager test | - | + |
| | | b. | Raymond test | ++ | + |
| | | c. | Legal test | + | ++ |
| | | d. | Baljet test | + | - |
| 6. | Tannins | a. | Gelatin test | ++ | + |
| | | b. | Phenazone test | ++ | ++ |
| | | c. | Match stick test | ++ | ++ |
| | | d. | Vanillin HCl test | + | +++ |
| 7. | Carbohydrates | a. | Molisch test | + | + |
| | | b. | Fehling test | + | + |
| | | c. | Benedict test | + | - |
| | | d. | Barfoed test | - | - |
| | | e. | Iodine test | + | + |
| 8. | Amino acids | a. | Millons test | + | ++ |
| | | b. | Ninhydrine test | + | ++ |
| | | c. | Biuret test | - | ++ |
| | | d. | Xanthoproteic test | - | ++ |
| 9. | Volatile oil | a. | Sudan III test | + | + |
| 10. | Fat and fixed oil | a. | Saponification test | - | - |
| 11. | Steriods & Terpenoids | a. | Libermann burchard test | ++ | ++ |
| | | b. | Sakowaski test | + | ++ |
| | | c. | Sulfur powder test | - | ++ |

Where: +ive indicates the presence and –ive indicates the absence of phytochemical constituent; + (low intensity of color), ++(medium intensity of color), +++(high intensity of color)

the presence of various chemical constituents like flavonoids, glycosides, alkaloids, steroids and saponins. Results are summarized in table 8.

**Physicochemical Investigation:** Results for Physicochemical Investigation are tabulated in Tablet 9.

**Estimation of Bioactive Constituents**

Bioactive constituent's estimation such **as** Total flavanoid content (TFC), Total phenolic content (TPC), Total tannin content (TTC) and Total Alkaloid Content (TAC) are tabulated in Tablet 10.

**Isolation and Characterization of plant extracts**

TLC chromatogram of MEIR, MECS was developed using solvent system BAW (4:1:5), BEW (4:1:2.2) with long and short U.V light and Iodine as detection reagents. The detection reagent showed few spots on TLC plates. The Rf value and of MEIR and MECS with inference are shown in Figure 4 and 5.

**Optimization of Chromatographic conditions:** Initial trials were conducted by using various mobile phases but final conditions were optimized by using conditions mentioned in material and method. Alantolactone and Proanthocyanidin were used as biomarker.

**Validation of Developed analytical method**

**Specificity:** The specificity was demonstrated by comparing the chromatograms of Reference and Test. From the study it was observed that both the markers are clearly resolved without any interference.

**System Suitability:** Confirmed that the given chromatographic conditions were good for method development and validation.

**Linearity:** Linearity data of photo markers are defined as for Alantolactone; $R^2$ Value is 0.9990 and equation Y= 118456.9 x -24463;

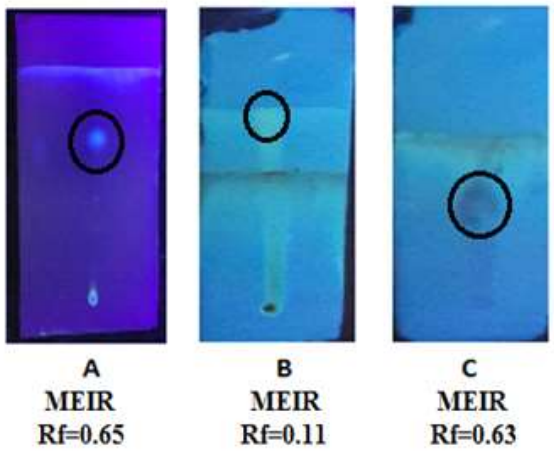


**Figure 4:** Slides represent TLC results for MEIR (Plate: TLC, silica gel 60), developing solvents: BAW (4:1:5), BEW (4:1:2.2), and detecting reagents: Long and short UV light. A. TLC plate for Rf value=0.65 i.e. Alantolactone, B. TLC plate for Rf value=0.11 i.e. Pyrogallol, C. TLC plate for Rf value=0.63 i.e. chlorogenic acid.

**Tablet 9:** Results of physico-chemical investigation of different extracts

| Parameters | *Inula racemosa* | *Ceratonia siliquila* |
|---|---|---|
| Total ash | 3.800% | 8.900% |
| Acid insoluble ash value | 0.300% | 1.600% |
| Acid soluble ash value | 1.500% | 2.400% |
| Alcohol soluble extract | 28.200% | 15.600% |
| Water soluble extract | 20.800% | 27.800% |
| Loss on drying (%M) | 7.800% | 5.560% |

**Table 10:** TFC, TPC, TTC & TAC of *Inula racemosa,* and *Ceratonia siliqua* extracts

| | | |
|---|---|---|
| **MEIR** | Total flavanoid content (mg QE/g) | 26.48±2.58 |
| | Total phenolic content (mg GAE/g) | 51.70±1.17 |
| | Total tannin content (mg TA/g) | 9.60±1.89 |
| | Total alkaloidal content (mg AE/g) | 21.64±2.96 |
| **MECS** | Total flavanoid content (mg QE/g) | 19.44±3.45 |
| | Total phenolic content (mg GAE/g) | 35.92±1.76 |
| | Total tannin content (mg TA/g) | 28.72±2.33 |
| | Total alkaloidal content (mg AE/g) | 17.82±1.54 |

Values are mean±SD with n=3

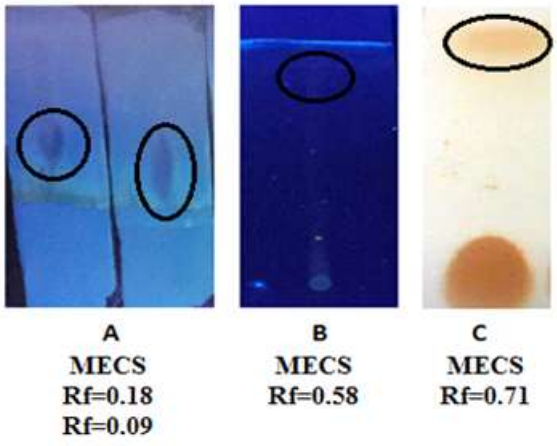


**Figure 5:** Slides represent TLC results for MECS (Plate: TLC, silica gel 60), developing solvents: BAW (4:1:5), BEW (4:1:2.2), and detecting reagents: Long and short UV light, Iodine reagent A. TLC plate for Rf value=0.18, 0.09 i.e. glycine, cysteine; B. TLC plate for Rf value=0.58 i.e. Malvidin (anthocynidine), C. TLC plate for Rf value=0.71 i.e. Quinic.

Proanthocyanidin; $R^2$ Value is 0.9993; and equation Y= 34998.4 x – 25113

**Accuracy:** The accuracy of the assay method, measured with the help of relative recovery at three different concentrations levels. All the values were with RSD ≤2%

**Robustness:** Performed by altering flow rate and Column temperature, all the results are within RSD ≤2%

**Results Analysis**

From the analyzed sample it was observed that the calculated assay content of alantolactone in *Inula racemosa* plant extract is 28.765% and in *Ceratonia siliqua* plant extract the calculated assay content of detected proanthocyanidin is 17.654%. All the assay values are calculated on anhydrous basis. Chromatograms are described in Figure 6.

**Excipient Compatibility Study:** Observations for compatibility study are tabulated in tablet indicate no such incompatibility in color or odor of excipients with plant extract signifies that the extracts are suitable with mentioned excipients. Thus these excipients are considered for the development of final formulation. Results of excipient compatibility study are tabulated in Table 11.

**Formulation Development**

**Responses observed in DOE model:** The design of Expert version: DX 13.0.12.0 is used in the present study for designing formulations. The two level factorial (2FI) model with three center points and subtype Randomized applies in which two independent variables i.e. binder Concentration (HPMC E-15) and Disintegrant Concentration (i.e. Crospovidone XL-10) at different levels were studied.

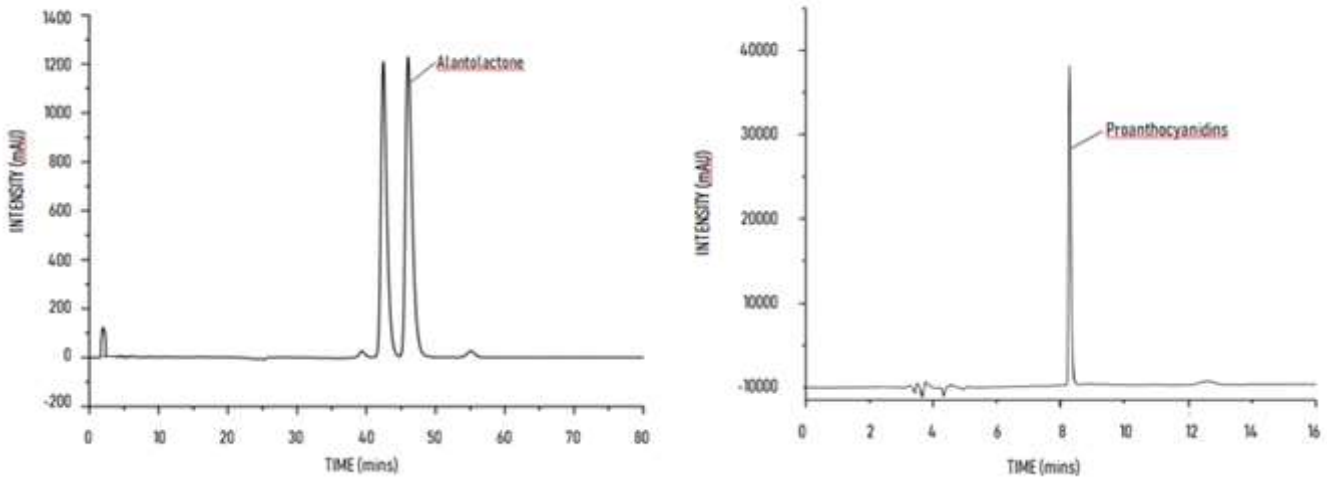


**Figure 6:** Representation of HPLC responses of a. Alantolactone in *Inula racemosa* b. Proanthocyanidin in *Ceratonia siliqua*.

**Tablet 11:** Results of Excipient compatibility (binary mixtures)

| S.No | Vial No. | Name of Ingredient | Controlled room temperature | | Open condition at 40 °C/75 % RH after 30 days | | Closed condition at 40 °C/75 % RH after 30 days | |
|---|---|---|---|---|---|---|---|---|
| | | | Color | Odor | Color | Odor | Color | Odor |
| 1 | 1A, 1B, 1C | *Inula racemosa* | Light Brown | Camphoraceous | Complies | No Change | Complies | No Change |
| 2 | 2A, 2B, 2C | *Ceratonia siliqua* | Dark Brown | Pungent | Complies | No Change | Complies | No Change |
| 3 | 3A, 3B, 3C | *Inula racemosa* + Microcrystalline Cellulose 200 | Light Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 4 | 4A, 4B, 4C | *Inula racemosa* + Crospovidone XL-10 | Light Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 5 | 5A, 5B, 5C | *Inula racemosa* + HPMC E-15 | Light Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 6 | 6A, 6B, 6C | *Inula racemosa* + Purified Talcum | Light Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 7 | 7A, 7B, 7C | *Inula racemosa* + Colloidal Silicon Dioxide | Light Brown with white spots | No particular Odor. | Complies | No Change | Complies | No Change |
| 8 | 8A, 8B, 8C | *Inula racemosa* + Magnesium Stearate | Light Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 9 | 9A, 9B, 9C | *Ceratonia siliqua* + Microcrystalline Cellulose 200 | Dark Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 10 | 10A, 10B, 10 C | *Ceratonia siliqua* + Crospovidone XL-10 | Dark Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 11 | 11A, 11B, 11C | *Ceratonia siliqua* + HPMC E-15 | Dark Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 12 | 12A, 12,B,12C | *Ceratonia siliqua* + Purified Talcum | Dark Brown | No particular Odor. | Complies | No Change | Complies | No Change |
| 13 | 13A, 13B, 13C | *Ceratonia siliqua* + Colloidal Silicon Dioxide | Dark Brown with white spots | No particular Odor. | Complies | No Change | Complies | No Change |
| 14 | 14A, 14B, 14C | *Ceratonia siliqua* + Magnesium Stearate | Dark Brown | No particular Odor. | Complies | No Change | Complies | No Change |

Based on this 6 different formulation at different Binder-Disintegrant ratio are designed. The results obtained are added to software against pre-predicted model values that shows the model is Significant. All the responses for all different Factors are recorded shown in table 12 and 13 for all pre and post compression parameters. Surface response of all DOE responses is shown in figure 7. As per the studied model P value in all the segments is less than 0.05 that indicate the model is significant for product development. Lack of fit test found to be bad that indicate that the model is fit of study. From the individual overlay plot draw against surface responses it was observed that the best result are obtained by F5 formulation that was reproduced in F6 and F7 is considered as optimized formulation for final product.

**Pre-compression studies**

From the observations it was observed that with increase and decreases in binder and disintegrat concentration no such major effect was observed on pre-compression parameters of the tablets. Observations of pre-compression study are tabulated in Table: 14.

**Post-compression studies**

From the results it was observed that with increase in binder concentration and decreased disintegrant concentration disintegration time of the tablet was directly get effected along with increased hardness, where as vice versa is observed at decreased binder and disintegrant concentration. Collective results for all trails are represented in Table 15.

**Table 12:** Representation of Responses against factors in DOE (Design of Experiment)

| Trial | Factor 1 A:Crospovidone XL-10 | Factor 2 B. HPMC E15 | Response1 Hardness (Kgf) | Response 2 Friability (%) | Response 3 Disintegration Time (min.sec) |
|---|---|---|---|---|---|
| F1 | 14.000 | 12.000 | 15.670 | 0.087 | 12.08 |
| F2 | 18.000 | 8.000 | 10.870 | 0.160 | 04.56 |
| F3 | 16.000 | 16.000 | 22.400 | 0.086 | 13.43 |
| F4 | 10.000 | 10.000 | 12.400 | 0.095 | 11.22 |
| F5 | **16.000** | **10.000** | **18.780** | **0.097** | **10.52** |
| F6 | **16.000** | **10.000** | **18.300** | **0.096** | **10.47** |
| F7 | **16.000** | **10.000** | **18.560** | **0.092** | **10.39** |

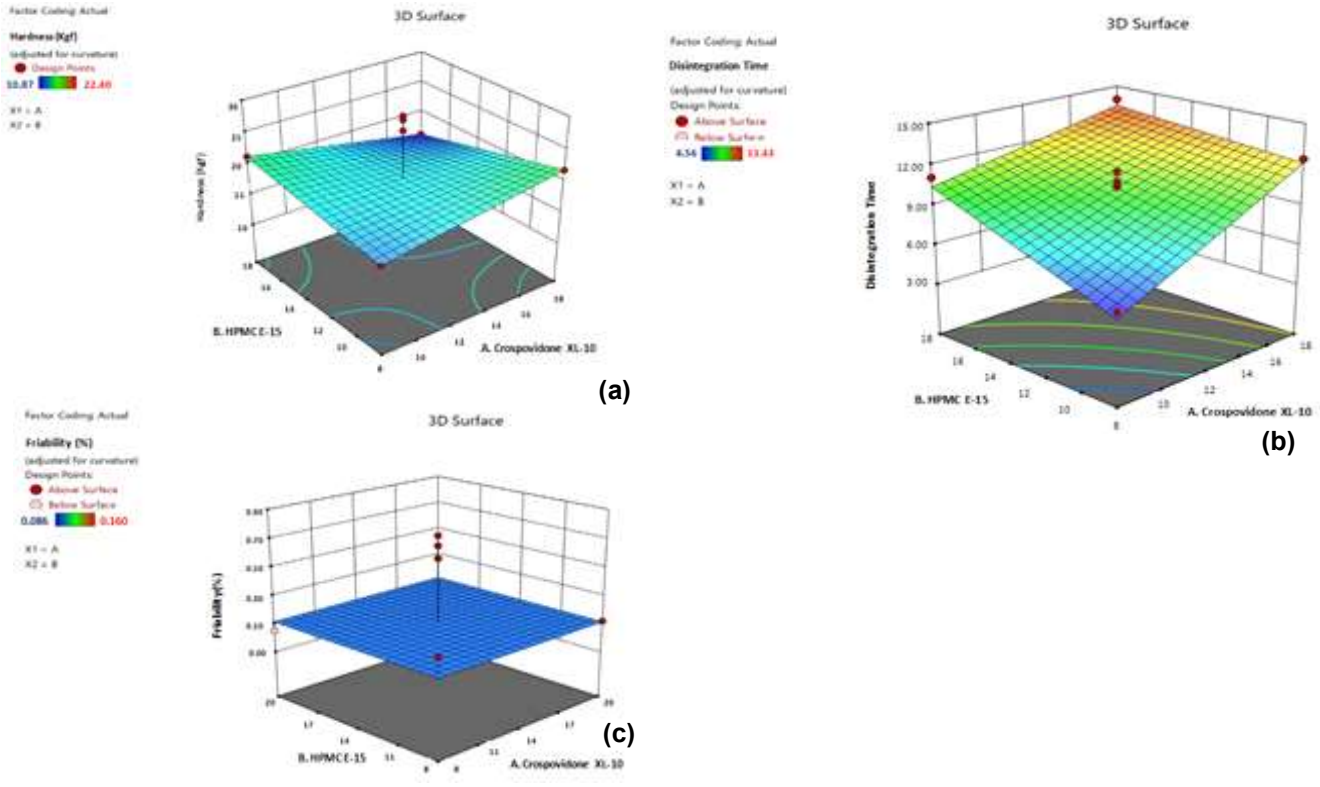


**Figure 7:** Representation of (**a)** Hardness Reponses, (**b)** Disintegration Time Reponses and (**c)** Friability responses for different designed formulations

**Tablet 13:** Representation of ANOVA Results for Factorial Model

| Response | Sum of squares | df | Mean Square | F-Value | Model p-Value | $R^2$ | Lack of Fit | Model |
|---|---|---|---|---|---|---|---|---|
| **Response 1** | 4.58 | 6 | 0.0721 | 11.87 | 0.0006 | 0.7912 | Bad (5.61) | Significant |
| **Response 2** | 1.65 | 6 | 0.0008 | 135.59 | < 0.0001 | 0.8047 | Bad (22.80) | Significant |
| **Response 3** | 9.870 | 6 | 0.0570 | 19.12 | < 0.0056 | 0.8765 | Bad (10.76) | Significant |

**Table 14:** Representation of Pre-compression studies

| Trial | Bulk Density (g/ml) | Tapped Density (g/ml) | Carr's Index (%) | Hausner Ratio | Loss at Drying (%M) |
|---|---|---|---|---|---|
| F1 | 0.563±0.0059 | 0.607±0.0008 | 7.249±0.034 | 1.0782±0.0087 | 5.670±0.045 |
| F2 | 0.571±0.0048 | 0.613±0.0067 | 6.852±0.078 | 1.0736±0.0067 | 5.809±0.0033 |
| F3 | 0.559±0.0056 | 0.599±0.0016 | 6.678±0.056 | 1.0716±0.0105 | 4.877±0.0023 |
| F4 | 0.574±0.0140 | 0.619±0.0080 | 7.269±0.067 | 1.0784±0.0123 | 5.023±0.0120 |
| F5 | 0.564±0.0050 | 0.609±0.0056 | 7.389±0.005 | 1.0798±0.0015 | 5.234±0.0214 |
| F6 | 0.577±0.0037 | 0.620±0.0075 | 7.167±0.0067 | 1.0745±0.0023 | 5.987±0.0063 |
| F7 | 0.557±0.0050 | 0.603±0.0076 | 7.629±0.0123 | 1.0826±0.0056 | 4.678±0.0077 |

**Tablet 15:** Observations of post-compression study

| Trial | Average Weight (mg) | Thickness (mm) | Dimension (mm) | Weight Variation (%) | Hardness (Kgf) | Friability (%) | Disintegration Time (min.sec) | Assay (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *Inula racemosa* | *Ceratonia Siliqua* |
| F1 | 650.000 | 5.032 | 16.50 x7.91 | ±1.476 | 15.670 | 0.087 | 12.08 | 27.65 | 20.40 |
| F2 | 648.000 | 5.035 | 16.52 x 7.91 | ±1.234 | 10.870 | 0.160 | 04.56 | 26.00 | 19.80 |
| F3 | 652.000 | 5.033 | 16.53 x 7.89 | ±1.345 | 22.400 | 0.086 | 13.43 | 29.78 | 19.60 |
| F4 | 653.000 | 5.032 | 16.50 x 7.01 | ±1.376 | 12.400 | 0.095 | 11.22 | 25.60 | 18.60 |
| F5 | 648.000 | 5.035 | 16.51 x 7.92 | ±1.434 | 18.780 | 0.097 | 10.52 | 28.60 | 18.40 |
| F6 | 650.000 | 5.034 | 16.53 x 7.92 | ±1.342 | 18.300 | 0.096 | 10.47 | 26.70 | 20.77 |
| F7 | 651.000 | 5.034 | 16.52 x 7.92 | ±1.432 | 18.560 | 0.092 | 10.39 | 27.70 | 19.54 |

### Stability studies

The analytical results of formulation F5, F6 & F7 formulation when compared to initial results, it was observed that there is no any degradation observed at $40^0$C temperature & 75% Relative humidity for 3 months. The formulation found stable.

### Conclusion

In current scenario the approach to ayurvedic drugs for weight management than preferring allopathic alternatives is emerging day by day. Herbal product may contain a single or combination of various herbs. Herbs in combination believed to have complementary or synergistic affects. The present study investigated studies involved in the formulating *Inula racemosa* and *Ceratonia Siliqua* tablets. The phyto-chemical as well as phyisco-chemical investigation were carried out prior to tablet development of *Inula racemosa* and *Ceratonia siliqua* tablets. The two level factorial (2FI) model design was applied by using design expert software. Six different formulations were studied at six different concentrations at variable Binder-Disintegrant ratios. Formulation F5 showed best result among all other formulations that were reproduced as formulation F6 and F7. The performed stability study also showed that the formulation is stable at $40^0$C temperature and 75%RH for 3 months. The whole study gives a brief idea and a support structure for developing *Inula racemosa* and *Ceratonia siliqua* plant extract tablets. In addition to that this study also provide information of possible studies involve in herbal tablet preparation.

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### Conflict of interest statement

The authors declare no conflict of interest.

### Author's contributions

All authors have contributed significantly to research concept, literature review, objectives and design of the study.

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