

FORMULATION OF POLYHERBOMINERAL MATRICES FOR TREATMENT OF OSTEOPOROSIS

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ABSTRACT

Objective: Herbal drugs are finding the new source of promising drugs for the treatment and cure of various ailments. The present research work deals with the formulation and evaluation of herbomineral formulation indicated for osteoporosis.

Materials and Methods: The aqueous extract of *Terminalia arjuna* (ARJ), *Asthisamhara* (ASTM), *Gokhru* (GKH), *Ashwagandha* (ASG) and dried powder of drugs *Sunthi* (ST), *Padmakashta* (PDMS), *Laksha* (PLSH) and *Jestamadha* were prepared and formulated into a herbomineral tablet with organic calcium viz.; *Godanti Bhasma* (Godan) and *Kukkutandatvak Bhasma* (Kukk) by wet granulation.

Results: The prepared tablets were evaluated for various parameters such as colour, average weight, hardness, friability and disintegration time, which were found acceptable as per pharmacopoeial specifications. The weight variation ($450 \text{ mg} \pm 10\%$), pH (4.58), disintegration time (17 min) and hardness (1.25 kg/cm^2) were found within acceptable range. The quantitative estimation of calcium by atomic absorbance spectrophotometry was carried out using ethanol-water solvent extraction method and found $76.27 \pm 0.439 \text{ mg/tab}$ of elemental calcium in optimized F4 formulation.

Conclusion: The developed herbomineral formulation is suitable for calcium deficiency by balancing hormonal imbalance and increases the rate of ossification of bone which helps to curing bone fracture, osteoporosis and calcium deficiency related problems.

Keywords: Polyherbomineral, *In-vitro* drug release, Principal component analysis.

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INTRODUCTION

Herbal medicines are in great demand globally for primary health care due to their higher safety margins and cost effectiveness. In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. WHO is involved in standardization and quality control of herbal crude drugs to monitor the physicochemical evaluation of crude drugs covering the aspects of the selection and handling of crude material, safety, efficacy, stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion [1,2].

Osteoporosis is a disease of the bones that leads to an increased risk of fracture. Osteoporosis is an important health-related problem that reduces the quality of life. Osteopenia is a condition where bone mineral density (BMD) is lower than normal. It is considered by many doctors to be a precursor to osteoporosis. More specifically, Osteopenia is defined as a BMD T scores - 1.0 to -2.5. Osteoporosis has been operationally defined on the basis of BMD assessment. According to the WHO criteria, osteoporosis is defined as a BMD that lies -2.5 standard deviation (SD) or more below the average value for young healthy women (T score of $< -2.5 \text{ SD}$). In other words, osteoporosis is defined as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture risk". In osteoporosis, there is a loss of bone tissue that leaves bones less dense and more likely to fracture. It can result in a loss of height, severe back pain, and change in one's posture. Osteoporosis can impair a person's ability to walk and can cause prolonged or permanent disability. In the United States, more than 40 million people either already have osteoporosis or are at high risk due to low bone mass [3-5].

The herbomineral drug is used for calcium deficiency in all the age groups, but mainly in childhood and elderly male and female. Calcium and physiotherapy were effective in improving mobility and pain. Calcium and Vitamin D3 group is effective to improve the total quality of life in osteoporotic patients [6]. Bone development is influenced by a number of factors, including nutrition, exposure to sunlight, hormonal secretions, and physical exercise. In the absence of this vitamin, calcium is poorly absorbed, the bone matrix is deficient in calcium, and the bones are likely to be deformed or very weak. Calcium is a mineral found in many foods. The body needs calcium to develop and maintain strong bones and to carry out many important functions. Almost all calcium is stored in bones and teeth, where it supports their structure and hardness. The body also needs calcium for muscles to move and for nerves to carry messages between the brain and every body part. In addition, calcium is used to help blood vessels move blood throughout the body and to help release hormones and enzymes that affect almost every function in the human body. Dietary supplements that contain only calcium or calcium with other nutrients such as Vitamin D are also available. The two main forms of calcium dietary supplements are carbonate and citrate. Calcium carbonate is inexpensive, but is absorbed best when taken with food. Calcium citrate, a more expensive form of the supplement is absorbed well on an empty or a full stomach. In addition, people with low levels of stomach acid absorb calcium citrate more easily than calcium carbonate. Other forms of calcium in supplements and fortified foods include gluconate, lactate, and phosphate [7].

Gokhru is stomachic, astringent, anthelmintic, but laxative large dose. Therefore, it is used in low appetite, piles and helminthiasis. Arjuna helps in wound healing and joining by astringent property. The bark paste is locally applied on wounds, ulcer and specially used in promoting the union of fractures. Ashwagandha used to reduce the oedema and pain which is applied on enlarged cervical glands or swelling of other glands in the form of root paste. *Asthisamhara* has haemostatic and joining properties [8]. Liquorice is a refrigerant, analgesic, anti-inflammatory

and helps hair growth. It is applied locally in poisoning and ulcerated wounds are healed with the local application. Padmakashta is helps in loss of appetite, laxity of stomach, vomiting, thirst, obesity. *Godanti Bhasma* is used in the treatment of leucorrhoea, headache, and fever due to pitta imbalance, chronic fever, cough, cold, asthma, anemia, chest injury, emaciation and wasting in children. It improves strength and immunity. *Zingiber officinale* is used to treat the problems related with gastrointestinal disorder which is effective improvement in general health such as anemia, body weight and gastrointestinal function and also improvement of absorption of calcium in blood [9]. *Godanti Bhasma* and *Kukkutandativak bhasma* are good calcium supplement to recover the calcium deficiency in child and adult. The *Godanti Bhasma* is very beneficial in the calcium deficiency and reduce the disease related to bones and also act as a calcium supplement to overcome calcium deficiency. Ayurveda provides many alternatives and can prove a boon to the humanity, not only by curing the disease, but also by preventing their recurrence. Keeping this in view, it becomes necessary to explore some curative, safe, and economical remedy, which can help the poor [10]. By considering market survey and literature review, the extract powders of an of *Terminalia arjuna*, *Asthisamhara*, *Gokhru*, *Ashwagandha* and dried powder of drugs *Sunthi*, *Padmakashta*, *Laksha* and *Jestamadha* along with *Godanti Bhasma* and *Kukkutandativak Bhasma* are effective in the treatment of osteoporosis. In this study, we developed a herbomineral formulation for treatment of osteoporosis and then characterized and conducted a test to obtain the suitability for effective tablets formulation for calcium deficiency by balancing hormonal imbalance and increases the rate of ossification of bone which help to cure bone fracture, osteoporosis and calcium deficiency related problems.

MATERIALS AND METHODS

Materials

T. arjuna, *Withania somnifera*, *Tribulus terrestris*, *Cissus quadrangularis*, *Z. officinale*, *Glycyrrhiza glabra* and *Butea Monosperma* were purchased from Wagh and Sons, Nagpur, Maharashtra. *Godanti* and *Kukkutandativak bhasma* were purchased from Jantayu Pharmacy, Jabalpur, Madhya Pradesh. All other ingredients such as starch, magnesium stearate and talc were purchased from Research-Lab Fine Chem. Industries, India. All ingredients used were of analytical grade.

Preformulation studies

The crude drugs dried and powder in cutter mill and grinder. The leaves were chopped and subjected to extraction using water as solvent as 60°C. The decoction was prepared by evaporating the extract to one third of its volume. The decoction was poured onto a glass tray and dried at 100°C. Dried extract was pulverized and stored in a desiccator.

Loss on drying

Exactly 2 g of each of the powder drugs sample was taken into hot and thin porcelain dishes and dried in the oven at 100°C. The dishes were then cooled in desiccators and loss in weight was recorded [11].

$$\text{LOD(\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (1)$$

Total ash value

About 2 g of powder drug was taken into the crucible and incinerated at a temperature not exceeding 450°C till it was free from carbon. The crucible was then cooled and weighed and the percentage of total ash was calculated with reference to air-dried drug powders [11].

$$\text{Total ash} = \frac{\text{weight of ash}}{\text{Weight of powder substance}} \times 100 \quad (2)$$

Acid insoluble ash value

The ash obtained as above was boiled for 5 min with 25 ml of dilute hydrochloric acid; the insoluble matter was hot water and collected on

an ash less filter paper, washed with ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Water soluble ash value

The ash obtained as above was boiled with 25 ml water for 5 min. All insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 min at the temperature not exceeding 450°C. The percentage of water-soluble ash was calculated by subtracting the weight of insoluble matter for weight of total ash. Percentage of water-soluble ash was calculated with reference to air dried drug.

Extractive values

The extractive values were obtained by exhausting crude drugs are indicative of approximate measure of their chemical constituents. Extracts were prepared with various solvent by standard methods. Percentage of dry extract was calculated in term of air dried powder drug part.

Water soluble extractive value

5 g of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 h, shaking frequently during 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a flat bottomed, shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as a % value.

Alcohol soluble extractive value

5 g of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for 24 h, shaking frequently during 6 h and allowing standing for 18 h. It was then filtered rapidly; taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed, shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air dried drug and is represented as a % value.

Inorganic constituents analysis

All extract and bhasma were analyzed for Aluminium, Chloride, Copper, Calcium, Carbonates and Bicarbonates, Magnesium, Nitrate, Phosphate, Potassium, Sodium, Sulphate, Zinc content using Inductively Coupled Plasma Mass Spectrometry. Individual ingredient was analyzed for arsenic content by Inductively Coupled Plasma Mass Spectrometry [12].

Phytochemical screening

The plant contains primary metabolite such as carbohydrates, proteins and lipids that are utilized as food by man, but also secondary metabolites like glycosides, alkaloids, volatile oil, tannin etc. that exerts physiological and therapeutic effects. Hence, plant material is subjected to preliminary phytochemical screening for detection of various chemical constituents using standard method [12].

Development of herbomineral matrix tablets

Herbomineral tablets prepared by using wet granulation technique. All the herbal ingredients were properly mixed with bhasma in a mortar to which strawberry flavor was added. This was followed by subsequent addition of starch and calcium phosphate dibasic and calcium carbonate (Table 1). After proper mixing of all the ingredients, sufficient quantity of distilled water was added to form a lumpy mass which was then passed through sieve No. 10 to form granules.

The granules were dried in the oven at 90°C for 5 h. The dried lumps were passed through sieve no. 12 to get appropriate granules. The granules were thoroughly mixed with magnesium stearate and compressed using 10 mm punch into a 450 mg tablet contains a number of herbs

Table 1: Compositions of herbomineral matrix tablet formulation

S.No.	Ingredients	Formulation code			
		F1	F2	F3	F4
1	<i>Tribulus terrestris terrestris</i>	65	65	65	65
2	<i>Terminalia arjuna</i>	50	50	50	50
3	<i>Withania somnifera</i>	40	40	40	40
4	<i>Kukkutandatvak Bhasma</i>	50	50	50	50
5	<i>Cissus quadrangularis</i>	40	40	40	40
6	<i>Zingiber officinale</i>	30	30	30	30
7	<i>Godanti Bhasma</i>	30	30	30	30
8	<i>Glycyrrhiza glabra</i>	30	30	30	30
9	<i>Butea Monosperma</i>	20	20	20	20
10	Calcium carbonate	20	25	25	25
11	Starch	20	25	30	35
12	Dibasic calcium phosphate	50	40	35	30
13	Magnesium stearate	5	5	5	5
14	Strawberry flavor	q.s.	q.s.	q.s.	q.s.

and mineral constituents using a single rotary punching machine (Cadmach Machinery, Ahmadabad).

Evaluation of herbomineral tablets

Pre-compression studies of powder blend

It is the principal investigation in the drug development to obtain information on the known properties of compounds and the proposed development schedule. So, this preformulation investigation may merely confirm that there are no significant barriers to compound development. Following pre-compression parameters were studied like the angle of repose, bulk density, tapped density, compressibility indices etc.

Angle of repose

It is the maximum angle that can be obtained between the freestanding surface of powder heap and the horizontal plane. It was determined by using fixed funnel method. Specified amount of powder drug was transferred to the funnel, keeping the orifice of the funnel blocked by thumb. When the powder was cleared from funnel, then measured its angle of repose and measured in θ [13].

$$\text{Angle of repose } (\theta) = \tan^{-1} h/r \quad (3)$$

Bulk density

It is the ratio of bulk mass of powder to the bulk volume. It is denoted by ρ_b . Bulk density is used to find out homogeneity.

$$\text{Bulk density } (\rho_b) = M/V_b \quad (4)$$

Where, M is the mass of the sample, V_b bulk volume.

Tapped density

It is the ratio of the weight of powder to the minimum volume occupied in measuring cylinder. Tapped density is determined by placing a graduated cylinder containing a known mass of drug or formulation on a mechanical tapper apparatus which is operated at fixed no. of taps until the powder bed reached a minimum volume [13].

$$\text{Tapped density } (\rho_t) = \text{Weight of powder blend} / \text{minimum volume occupied by cylinder} \quad (5)$$

Carr's index

Based on the apparent bulk density and the tapped density, the percentage compressibility of the powder mixture was determined by the following formula [13].

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \quad (6)$$

Hausner's ratio

It is an indirect index of ease of measuring of powder flow. Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25)

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{bulk density}} \quad (7)$$

Moisture content

Place about 10 g of granules in tarred evaporating dish and place it in oven at 105°C for 5 hrs and weigh. Continue the drying and weighing at 1 h interval until the difference between two successive weighing corresponds to NMT 0.25%. Weigh the sample till constant weight is obtained [13].

Post-compression studies of prepared herbomineral tablets

The herbomineral tablets were evaluated for various parameters like appearance, thickness, weight variation, hardness and friability [14].

Physical appearance

The general appearance of tablet was studied visually in shape, color, texture and odour.

Thickness

The tablet thickness was calculated by Vernier calipers. The tablet was put in between two jaws vertically and measured thickness and 6 tablets were used for this test and expressed in mm.

Weight variation

Randomly selected 20 tablets were weighed individually, calculating the average weight and comparing individual tablet weight to the average. The weight variation test would be a satisfactory method of determining the drug content uniformity of tablets.

Hardness

The tablet hardness was determined by the Monsanto hardness tester. The tablet was placed lengthwise between upper and lower plunger and force applied by turning a threaded bolt until the tablet fractures and measured hardness of tablet in kg/cm^2 .

Friability

It is determined by Roche friabilator, subjects a number of tablets to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, dropping tablet from inches distance operated for 100 revolutions [13]. The pre weighed tablets were dusted and reweighed and according to standard limit friability should be $<1\%$. It is calculated by formula;

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (8)$$

Disintegration time

Disintegration test was performed according to the Indian Pharmacopoeia specification. Six tablets were taken for the test and water was as the disintegration medium. The temperature of the medium was kept at 37°C , the beakers were filled at a volume of 800 ml and care was taken that the tablets were always below the level of the water at the highest and lowest position of basket-rack assembly. The discs were introduced over each tablet to avoid their floating of the tablets in the medium. The apparatus was operated until all the tablets were disintegrated [14].

Preparation of tablet samples for calcium analysis with the ethanol-water solvent

About 0.5 g of the dried finely ground tablet material (ca. 30–60 mesh) was refluxed for 15–30 min in a round bottomed flask with about 35 ml of 20% v/v ethanol-water mixture. The resulting solution of activated carbon and filtered directly into a 50 ml volumetric flask,

through Whatman No. 42 filter paper. The filter was washed with the solvent and the filtrate was received in the same volumetric flask. The volume of the combined filtrates was completed with the same ethanol-water mixture. With these solutions, adequate aliquots (10.0 ml) were taken and diluted to 50.0 ml to determine calcium using calibration curves [15].

Content uniformity for calcium

A stock solution of calcium standard (1000 mg/l) was prepared by dissolving 2.4973 g of dry CaCO₃ in 200 ml of distilled water containing 5 ml of concentrated HCl. The solution was heated to drive out CO₂ and after cooling, it was made up to 1000 ml. The stock was diluted to produce working standards of concentration range of 1–100 µg/ml. Lanthanum (0.1%) was used in these solutions, and the same procedure was used in plant samples, to avoid interference of phosphorus. 1.0 ml of the ethanol-water mixture was added to the final solutions. The AAS was set at the operating wavelength of calcium. A Carl Zeiss FMD/PMQ3 atomic absorption spectrophotometer with a single element hollow cathode lamp, a 10 cm universal burner and acetylene air flame, was used for measurements of calcium at 422.7 nm. Each working standard was run in the emission spectrometer and the intensity for each standard was recorded [15]. The calibration curve was constructed to determine the concentration of calcium in the tablets sample. The blanks were also determined in the same way and subtraction done where necessary.

$$\text{Calculations: Ca (mg/kg)} = (a-b) \times V \times f \times 1000 / 1000 \times w \quad (9)$$

Where: A=Concentration of Ca in the sample extract b=Concentration of element in the blank extract P v=volume of the extract solution w=weight of the soil sample f=dilution factor.

Principal component analysis (PCA) of variables in formulation

PCA was adopted using XLSTAT 2017 to analyze the variations in physicochemical parameters of both samples. PCA is the most widely used multivariate analysis technique for transforming the original measurement variables into new variables called PCs. Each PC is a linear combination of the original measured variables. It is possible to identify key relationships in the data that is, find similarities and differences among objects in a data [16].

Stability studies

The optimized formulation was subjected to stability at 40±2°C and 75±5% RH for a period of 6 months. After each month tablet sample was analyzed for physical characteristics and disintegration time [17].

RESULTS AND DISCUSSION

It was observed that total ash value was found to be very high in Gokshura and Asthisamhara. Ash values are the criteria to judge the identity and purity of crude drugs, where total, water soluble and acid insoluble ashes are considered. All the parameters were found to comply with the standards. This formulation was evaluated for different parameters like moisture content, ash value and extractive value, result shown in Table 2. All the components were found to be within specified limits.

Inorganic constituents present in herbs and bhasma are calcium, magnesium, phosphate, potassium, chloride, nitrate and sulphate. Phytochemical screening was carried out according to standard methods. These parameters were compared with standard values, and were found to be within the specified limits, all the results shown in Table 3. Excipients profile starch takes up water from the body fluids which cause it to swell and thereby leading to the disintegration of the granules. Calcium phosphate dibasic and calcium carbonate were used as bulking agents. Magnesium stearate helps to prevent attrition between the granules and formation of fines. The flavoring agent helps to mask the bitter taste of crude powder. As they are triturated with the drug at the very beginning of the preparation before the addition of other excipients, they form a coating over the drug particles and hence in spite of disintegration within the oral cavity it makes the formulation highly palatable.

The extract of Arjuna, Asthisamhara, Gokhru, Ashwagandha and dried powder of drugs sunthi, Padmakashta, Laksha, Jestamadha and Kukkutandatwak bhasma, Godhanti bhasma were passed through 60 no. sieve, it is kept in drier at 90°C for 5–6 h, dry the granules, after that compressed it.

Pre compression studies of powder blend

The granules were prepared by using dried extract of herbs and bhasma. The powder blend was evaluated for various parameters and their results are shown in Table 4. The moisture content of F4 was less than the other which is one of the main parameters that determines the shelf life of a product. Moisture content is the main causative factor in the deterioration. Moisture in a product is sufficient to activate different enzymes, which slowly decompose the product resulting in its degradation [18]. After evaluation of preformulation parameters, it showed that there is no presence of moisture in powder and showed uniformity of powder blend. The evaluation parameters such as angle of repose, bulk density, tapped density; Carr's index and Hausner's ratio were found to be 23.72±0.11–30.40±0.12 (θ),

Table 2: Preliminary phytochemical and physicochemical parameters

Parameters	ARJ	PDMS	ST	PLSH	ASG	LQR	GKH	ASTM
Carbohydrate	+	+	+	+	+	+	+	+
Protein	-	-	-	+	-	-	-	+
Amino acids	+	-	+	-	+	-	+	+
Glycosides	+	+	+	+	+	+	+	-
Flavonoids	+	+	-	-	-	+	+	+
Alkaloids	+	+	-	-	+	-	+	-
Steroids and triterpenoids	+	-	-	-	+	+	+	+
Tannins and phenolics	+	+	-	-	+	+	+	+
Gums	+	+	+	+	+	+	+	+
Fixed oils	-	-	-	-	-	-	+	-
Volatile oils	-	-	+	-	-	-	-	-
Mucilage	-	-	+	+	-	-	-	-
Saponine	+	+	-	-	+	+	+	-
Loss on drying*	20	10.15	4.2	9.4	11.3	2.85	9.2	75.12
Total ash*	8.5	0.4	3.85	2	5	6	10	16
Acid-insoluble ash*	0.5	0.3	1.9	0.3	0.2	1	1.5	20
Water-soluble ash**	2	0.5	1.7	1	2.7	7.12	3.4	2
Water soluble extractive value**	25	4	12	65	13.5	25	20	25
Alcohol soluble extractive value**	28	5	4.89	75	17	30	10	8

*NMT: Not more than, **NLT: Not less than, "+" indicates present and "-" indicate absent

0.42±0.02–0.625±0.012 g/ml, 0.38±0.031–0.555±0.012 g/ml, 11.2±0.441–17.1±0.03, 1.2±0.012–1.5±0.13 respectively. The values of the angle of repose are below 30° thereby indicating excellent flow properties. Lower values of bulk and tapped density indicate higher porosity implying the time required for disintegration would be lower. % LOD test values comply with the official limits and indicate lower moisture content in the formulation. After study of flow rate it concludes that powder blend exists optimum proportion that leads to the maximum flow rate. So the result showed that the powder has good flowing property which does not cause affect the process of tablet punching [13]. The physiochemical property show satisfactory results by herbomineral tablet which are within the range of prescribed standards required for investigation of present study.

Post compression parameters

Herbomineral tablet is formulation intended to be swallowed and to release the drug in a relative short time, thereafter by disintegration and dissolution to overcome the calcium deficiency, osteoporosis, bone fracture, bone pain, rapid wound healing and to balance hormone such as testosterone with the help of herb and mineral which contain abundant amount of calcium element which correct calcium deficiency and related problems. The result from different physical parameters like thickness, hardness, weight variation, and friability of tablets was shown in Table 5. The general appearance of the tablet was found to be round in shape, brown in color, smooth texture, and odorless. The thickness, hardness, weight variation, and friability of herbomineral tablet were founded to be in acceptable limit.

All the herbomineral tablets passed the weight variation test as the average percentage weight variation was within the USP limits of ±5%. The weight variation of tablet causes variation of active medicament which changes the bioavailability. This may be due to causes such as variation in granule size, poor flow, bridging, rat holing, punch variation and poor mixing. Herbomineral tablets were found to have (mean±SD) 450 mg average weight. 90% tablets were within acceptable range of weight variation as for natural herbal products, ±5% range of weight variation is acceptable. The hardness of conventional herbomineral tablet was found to be 4.31±0.2–9.0±0.30 kg/cm². Tablets showed sufficiently hard to resist breaking during packaging, shipment, and normal handling. Friability of all formulations was found to be 0.243±0.063–0.405±0.305%.The friability of tablet was found to be in acceptable limit i.e. <1%. There no capping problem occurs in the tablets so it could be considered for commercial use. It produced no loss during the shipping process. The disintegration test implies that the granules can disintegrate within 20 min, thereby leading to quicker absorption and onset of action of the drug. It shows that the herbal drugs containing herbomineral tablets have satisfactory disintegration profile due to their hardness within range of standard limit [15].

Quantitative estimation of calcium by atomic absorbance spectrophotometry

The calibration curve for quantitative estimation of calcium by atomic absorption spectrophotometer is shown in Fig. 1. The quantitative estimation of calcium by atomic absorbance spectrophotometry was carried out using ethanol-water solvent extraction method and found

Table 3: Qualitative test for inorganic constituents

Elements	GKH	ARJ	ASG	ASTM	ST	LQR	PLSH	PDMS	Kukk	Godan
Alluminium	-	-	-	-	-	-	-	-	-	-
Chloride	+	-	+	-	+	-	+	-	+	+
Copper	-	-	-	-	-	-	-	-	-	-
Calcium	-	+	-	-	-	+	-	-	+	+
Carbonate and bicarbonate	-	-	-	-	-	-	-	-	-	-
Magnesium	-	+	-	-	-	-	-	-	-	-
Nitrate	+	-	+	+	-	-	-	-	+	+
Phosphate	+	+	-	+	+	-	-	-	+	+
Potassium	-	-	-	-	-	+	-	-	-	-
Sodium	-	-	-	-	-	-	-	-	-	-
Sulphate	-	-	-	-	-	-	-	-	-	+
Zinc	-	-	-	-	-	-	-	-	-	-

*NMT: Not more than, **NLT: Not less than, "+" indicates present and "-" Indicate absent

Table 4: Precompression studies trial batches

Pre-compression parameters	F1	F2	F3	F4
LBD (g/ml)	0.625±0.002	0.42±0.04	0.42±0.02	0.625±0.011
Tapped density (g/ml)	0.526±0.04	0.38±0.031	0.39±0.012	0.555±0.015
Carr's index	15.84±0.271	17.1±0.05	16.4±0.34	11.2±0.441
Hausner's ratio	1.3	1.5	1.4	1.2
Angle of repose (θ)	26.20°	30.40°	28.25°	23.72°
Moisture content (%)	3.50%	4.00%	6.20%	2.5%

LBD: Loose bulk density

Table 5: Post-compression studies of herbomineral tablets

Parameter	F1	F2	F3	F4
Colour	Brown	Brown	Brown	Brown
Diameter	10.90±0.20	10.50±0.20	10.30±0.20	10.89±0.20
Thickness (mm)	5.8	6.90	6.80	6.6
Disintegration time (min)	26.20±0.30	45.10±0.25	40.20±0.30	25.19±0.20
Friability (%)	0.263±0.02	0.405±0.30	0.302±0.203	0.243±0.063
Hardness (kg/cm ²)	9.0±0.30	8.0±0.10	7.0±0.40	7.0±0.24
pH	8.0	9.7	10.0	9.4
Average weight (mg±SD)	448±0.40	450±0.50	448±0.10	451±0.20

SD: Standard deviation

Table 6: Proximity matrix (n)

Matrix	F1	F2	F3	F4
F1	86.099	115.430	103.632	72.816
F2	115.430	160.588	142.929	97.391
F3	103.632	142.929	128.157	87.407
F4	72.816	97.391	87.407	63.110

Table 7: Squared cosines of the variables

Variables	F1	F2	F3	F4
Variable 1	0.987	0.009	0.004	0.001
Variable 2	0.993	0.006	0.001	0.001
Variable 3	0.995	0.002	0.000	0.002
Variable 4	0.966	0.029	0.004	0.000

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

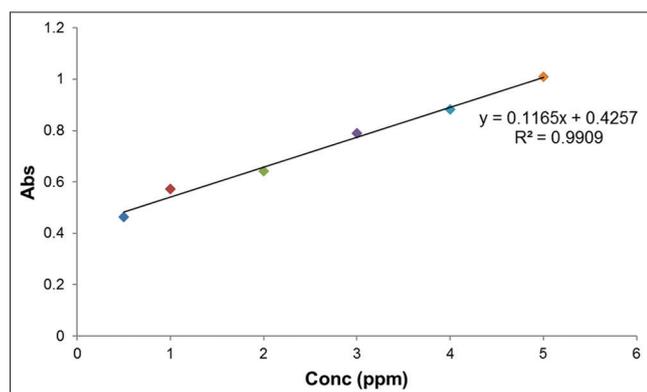


Fig. 1: Calibration curve for calcium standard

53.57±0.895, 62.0±1.198, 65.06±1.074 and 76.27±0.439mg/tab of elemental calcium in F1, F2, F3 and F4 respectively.

PCA

PCA was adopted using XLSTAT 2017 to analyze the variations in physico chemical parameters of all formulations (Table 6). It is possible to identify key relationships in the data that is, find similarities and differences among objects in a data. PC1 shows more residual x-variance in variables given in Table 7.

Using PCA it is found that all data can be described with PC1, hence discrete variations were found in samples and the data can be easily explained with a multivariate model given in Fig. 2.

Stability studies

The stability of herbal products depends on the various factors such as stability of herbal ingredients, manufacturing processes, the reaction between active herbal ingredients and excipients, containers, environmental conditions encountered during storage and microbe's etc. Accelerated stability testing based on the single condition of elevated temperature and humidity is more appropriate and suitable for herbal products because of their very basic nature. The optimized batch (F4) was subjected to the accelerated storage condition (40°C/75% RH) for 6 months according to ICH guideline. The results shows stable with no significant changes in the physio-chemical properties of the tablets as well as no remarkable changes in the release profile (Fig. 3).

CONCLUSIONS

From the above study, we conclude that the herbomineral tablets were prepared by the wet granulation method and gave satisfactory and acceptable results. The developed herbomineral formulation from *T. terrestris*, *T. arjuna*, *W. somnifera*, *C. quadrangularis*, *Prunus cerasoides*, *G. glabra*, *Butea Monosperma*, *Z. officinale* and *Godanti Bhasma* and *Kukkutanta twak bhasma* is a better formulation for calcium deficiency

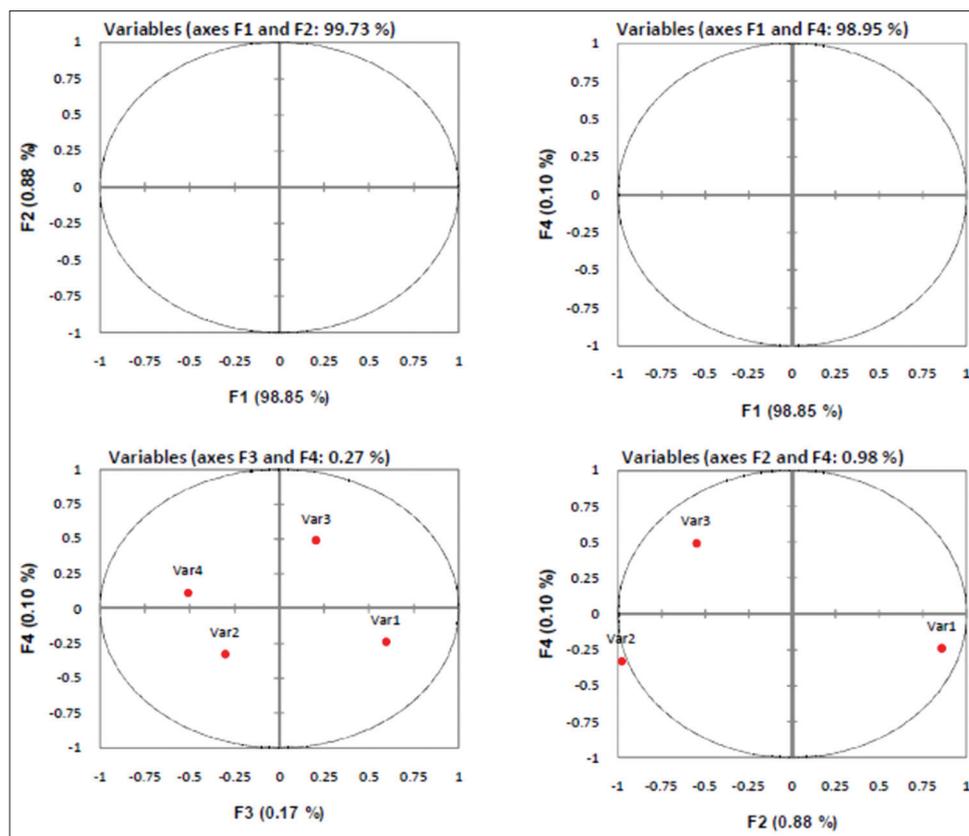


Fig. 2: Variable for correlation shows multivariant

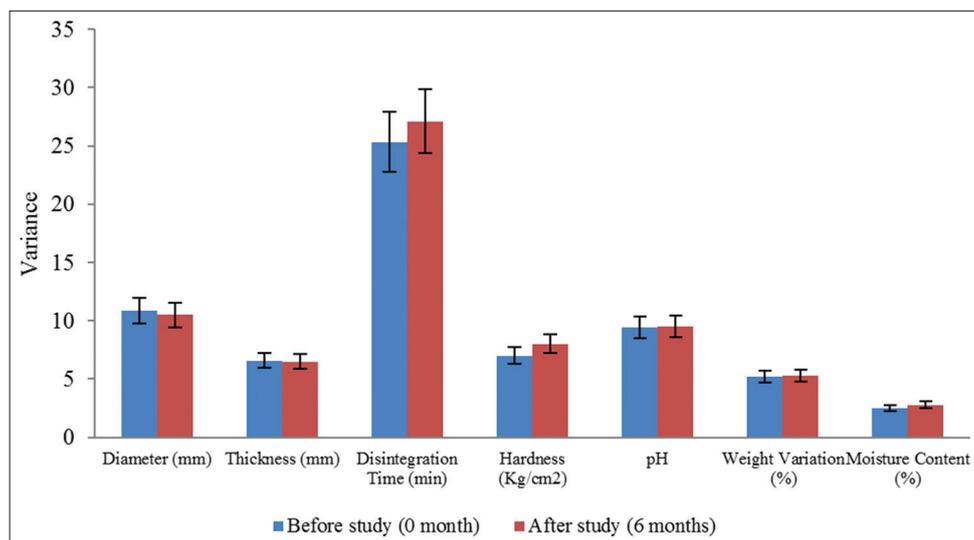


Fig. 3: Stability for optimized F4 formulation

by balancing hormonal imbalance and increases the rate of ossification of bone which help to cure bone fracture, osteoporosis and calcium deficiency related problems. However, further research is needed to study their activity clinically and to study their precise mechanism of action and efficacy with long term use as a calcium supplement to rectify the calcium deficiency.

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