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Formulation and Evaluation of Poly Herbal Tablet for Bone Healing

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Abstract

The present study was designated to formulate and evaluate the poly herbal tablet for bone healing properties. The plants were selected on the basis of their individual properties for restoring fractured bone to its original along with the supporting actions of anti-inflammatory, calcium supplements and others numbers of nutrients and supplements, analgesic, wound healing, anti-arthritic activities for the better and faster reformation of bone. Plants such as hadjod, guggul, babool, triphala and ashwagandha used respectively. Babool helps us giving calcium supplement, guggul increases deposition of calcium towards bone, triphala shows wound healing properties and ashwagandha is used for its analgesic activities. The doses were selected on the basis of referencing material along with the upper and lower limit of usage of dose. The three formulations of varying amount of composition are prepared and evaluation is carried out. The physical evaluation parameters were performed for the tablet formulation. The pre as well as post evaluation parameters were observed, and result were calculated, and comparison studies are carried out to find out the optimum formulation.

Keywords

Fracture, Bone healing, Hadjod, Guggul, Babool, Triphala, Ashwagandha, Bone restoration, cell proliferation and differentiation, calcium supplements, anti-inflammatory, analgesics, wound healing

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1. Introduction

- Fractures are complete or incomplete separations in bone continuity.
- Hematopoietic and immune cells in bone marrow work together to heal fractures, a complex process.
- Bone healing, also known as fracture healing, is a physiological process that helps the body repair bone fractures. It is a complex process involving cell and tissue proliferation and differentiation.
- Growth factors, inflammatory cytokines, anti-oxidants, osteoclasts and osteoblasts, hormones, amino acids, and uncounted nutrients all play a role.
- Periosteum plays a crucial role in the healing process. The periosteum is a source of precursor cells that develop into chondroblasts and osteoblasts, which are necessary for bone healing [1].
- To improve fracture healing, two options are implant development and bone quality improvement to accelerate callus formation [2].
- Fracture healing involves three stages:
- The reactive phase includes fracture and inflammation, followed by granulation.
- Reparative phase includes cartilage callus formation and lamellar bone deposition.
- During the remodelling phase, return to the original bone counter.

1.1. Inflammatory Stage

When a bone fractures, the body sends signals to bring special cells to the injured area. Some of these special cells cause inflammation in the injured area (redness, swelling, and pain). This instructs the body to stop using the injured part in order for it to heal properly. During this stage, additional cells form a hematoma (blood clot) around the broken bone. This is the first connection between the broken bones.

1.2. Reparative Stage

The reparative stage begins within a week of the injury. A soft callus (a type of soft bone) replaces the blood clot that formed during the inflammatory process. The callus holds the bone together but is not strong enough to allow the body part to function. Over the next few weeks, the soft callus will harden. This hard callus becomes strong enough to allow the body part to be used after about 2-6 weeks.

1.3. Remodelling Stage

The remodelling stage begins approximately 6 weeks after the injury. During this stage, regular bone replaces the hard callus. If you saw an X-ray of the healing bone, it would appear uneven. However, over the next few months, the bone is reshaped to resemble its pre-injury appearance.

Anti-inflammation is a key principle in treating fractures.

Reduces swelling and pain at the fracture site and surrounding tissues, promoting healing through increased production of mediators like arachidonic acid and cytokines. Inducible nitric oxide synthase (iNOS) produces nitric oxide (NO), which has been linked to inflammatory disorders like rheumatoid arthritis.

Table 1. Drug Profile.

Hadjod [Cissus Quadrangularis]  **Pharmacological activity:** Ossification and remodeling of bones, acts by stimulating metabolism and increasing the uptake of calcium, sulphur, and strontium by osteoblasts in fracture healing[6].	**Botany:** Cissus quadrangularis or 'Hadjod' as it is known in Hindi, is a fleshy plant of the Vitaceae family that can be found throughout India[5]. **Powder:** Brown in color with pieces of fibers, vessels, parenchymatous cells, and a few calcium oxalate rosette crystals. It also contains starch grains, an idioblast that contains raphides, and isolated calcium oxalate acicular crystals[6]. **Chemical constituents[7]** Calcium Oxalate, Carotene Ascorbic Acid Iridoids **Benefits[6]** Fracture healing, Anti-oxidant, Anti-ulcer, Boost weight loss, Anti-tumor, Reduce risk of type2 Diabetes, Reduce Heart diseases
Guggul [Commiphora WeightII]  **Pharmacology:** There are notable anti-inflammatory and anti-arthritic properties in the oleoresin fraction of guggul. It increases the deposition of calcium towards the bone[9].	**Theory:** The family Burseraceae includes the oleo-gum resin known as guggul, which is extracted from the bark of the wight tree. It is extracted as an exudate by tapping the guggul tree's stems and branches[8]. **Chemical Composition[8]:** Complex mixture of steroids Guggulsterol [I,II,III,IV,V] Guggul sterone Diterpenoids Triterpenes **Therapeutic uses[10]:** Anti-inflammatory Rheumatoid Arthritis Anti-atherosclerosis Anti-obesity Wound healing

Triphala [Amalaki, Haritaki, Bibhitaki]

Theory: According to the Ayurvedic Formulary of India, triphala is a blend of three fruits made up of the dried fruits of Emblica officinalis Gaertn (Euphorbiaceae), Terminalia belerica Linn (Combertaceae), and Terminalia chebula (Combertaceae) in equal amounts (1:1:1).

Rich source of Vitamin C, flavonoids, ellagic acid, gallic acid, chebulinic acid, and ascorbic acid are among the chemical constituents. Approximately 20 percent of triphala also consists of hydrolysable and condensed tannins[11].

Drugs	Botany	Chemical Constituents	Therapeutic Uses[13]
Amalaki [Emblica Officinalis][12]	Ripe fruits take on a light brown color, while fresh fruits have a light green hue. The fruit weighs between 60 and 70 grams on average.	Vitamin C Tannins [Gallic Acid, Ellagic Acid, Phyllembin] Minerals Amino acid	Anti-Inflammatory Analgesic Anti-Pyretic Anti-Hyperlipidimic Immune modulatory effect Wound healing Anti-Bacterial Anti-Diabetic
Haritaki [Terminalia Chebulalinn][13]	The fruit is blackish in color, drupe-like, and measures 2-4cm (0.79-1.77in) in length and 1.2-2.5cm (0.47-0.98 in) in width. It has five longitudinal ridges.	Arjunglucoside Arjugenin Chebuloside [I,II] Chebulin	
Bibhitaki [Terminalia Bellerica Roxb][13]	The nuts of the tree are rounded but with five flatter sides. The leaves are about 15 cm long and crowded toward the ends of the branches.	Tannins Gallo-tannic acid Resins	

Ashwagandha [Withania Somnifera]

Pharmacology:

Anti-inflammatory/anti-arthritic/analgesic activities:

Effective inhibitors of cyclooxygenase-mediated arachidonic acid metabolism which results in the production of prostaglandins that cause erythema, pain and edema are anti-inflammatory phytomedicines[15].

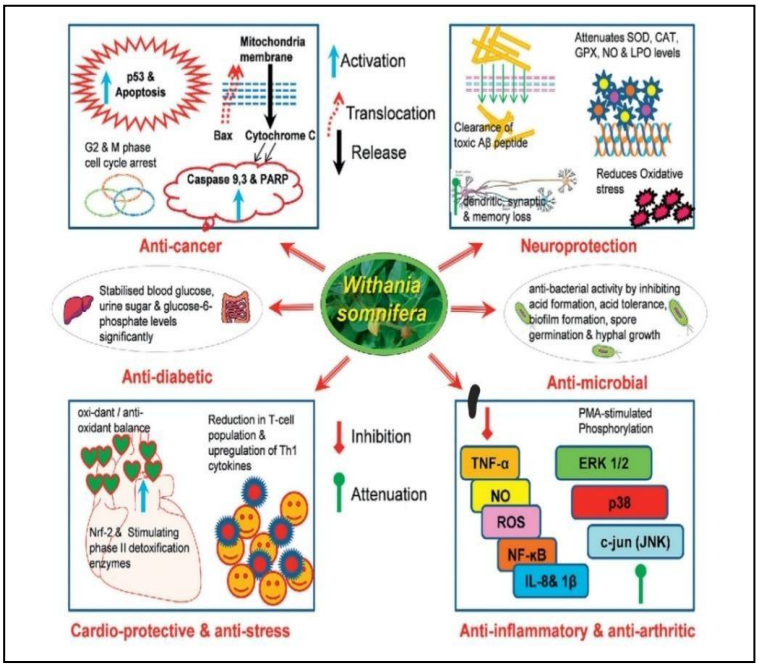
Theory: Common names for Withania somnifera (WS) include winter cherry, Indian ginseng, and ashwagandha.The primary parts of the plant that are used medicinally are the roots[14].

Chemical Composition [15]:

Alkaloids (isopellertierine, anferine),

Steroid lactones (as in withanolides and withaferins).

Iron is also abundant in Withania somnifera. The main substances found in Withania somnifera roots are called withanolides.



Babool [Acacia Arabica]

Pharmacological Action[18]

Both they and their inhibitory effects on osteoclastogenesis are positive. These extracts therefore hold promise for the development of osteoporosis and bone fracture therapies.

Botany: Acacia is the most important genus in the Leguminosae family, having been identified by Linnaeus. Acacia species are well known in India as 'Babool' and have been traditionally used in treatment of skin, BONES, stomach, genital and dental problems [16].

Chemical Constituent: [17]

Bark: Gallic acid, phlobatannin, and condensed tannin. It has a tannin content of 12–20%.

Gum: Four aldobiouronic acids, galactose, L- rhamnose, and L-arabinose are all present in gum. Fruits: Phenolic components include saponins, gallic acid, mucilage, and m-digallic acid. 32% of it is tannin-filled.

Flowers and pods: Stearic acid and kaempferol-3- glucoside are found in flowers. Tanning beans have both oxalic acid and tannin.

Leaves: 32% tannin, rutin, apigenin, and 6-8-bis- D-glucoside are present.

Inflammatory-reducing properties[19]

Studies on the yellow pigment's ability to reduce inflammation both acutely and over time have shown encouraging results.

Additionally, a significant percentage of the bacteriostatic effect was protected.

1.4. Rationale

It takes more than a single plant's active phytochemical components to produce the intended medicinal effects. The toxicity will be decreased and the therapeutic effect will be improved when different herbs are combined in a specific ratio.

Table 2. Drug Rationale.

Sr. no.	Drugs	Pharmacological Action
1	Hadjod	Calcium, sulphur, and strontium uptake by osteoblasts is enhanced when their metabolism is stimulated during fracture healing.
2	Guggul	Significant anti-arthritic, anti-obesity and anti- inflammatory activities, deposition of calcium on bone.
3	Triphala	Shows anti-inflammatory, analgesic, wound healing and anti-arthritic activity.
4	Ashwagandha	Inhibit arachidonic acid metabolism > produce no prostaglandin > no pain and oedema.
5	Babula tweak	Increase bone cell proliferation, have a favorable impact on osteogenesis, and suppress osteoclastogenesis.

1.5. Aim

To Formulate and Evaluate Poly- Herbal Tablet for Bone Healing.

1.6. Objective

- To determine higher efficacy for Remodelling of Bone.
- To estimate anti-inflammatory and analgesic activity.
- To evaluate wound healing effect.
- To determine the stability of poly herbal tablet.

2. Materials and Methods

2.1. Formulation for Tablet [24]

In this poly herbal tablet formulation the dried powder of Hadjod [Cissus Quadrangularis], Guggul [Commiphora WeightII], Triphala [Amalaki, Haritaki, Bibhitaki], Ashwagandha [Withania Somnifera], Babool [Acacia Arabica] along with other excipients was formulated in tablet dosage form via wet granulation technique.

Table 3. Formulation for Tablet.

DRUGS	F1	F2	F3
Hadjod	1 part	1.5 part	2 part
Babool	1 part	1 part	1 part
Triphala	1 part	0.5 part	0.4 part
Ashwagandha	1 part	0.5 part	0.4 part
Guggul	1 parts	1.5 part	1.2 part

Table 4. Composition for Tablet.

Sr.No.	Ingredients	F1	F2	F3	Standard Range
1	Cissus Quadrangularis	70mg	105mg	140mg	-
2	Acacia Arabica	70mg	70mg	70mg	-
3	Triphala	70mg	35mg	28mg	-
4	Withenia Somnifera	70mg	35mg	28mg	-

5	Commiphora Wightii	70mg	105mg	84mg	-
6	Honey (binder)	Qs	Qs	Qs	-
7	Lactose (filler)	20mg (5%)	20mg (5%)	20mg (5%)	5% - 20%
8	Talc (glidant)	8mg (2%)	12mg (3%)	16mg (4%)	2% - 5%
9	MCC (super disintegrant)	2.0mg (0.5%)	2.8mg (0.7%)	3.6mg (0.9%)	0.2% - 2.0%
	Total	400 mg	400mg	400 mg	-

2.2. Procedure

2.2.1. Weighing of Ingredients

All crude ingredients firstly ground into fine powder and should be passed through sieve no. 80 mesh. It must be weighed accurately by using weighing balance with assured calibration.

2.2.2. Mixing the Powder Ingredients and Excipients

All the excipients and powdered API should be mixed in ascending order of their weight. It should be mixed properly, to prepare a homogenous mixture for manufacturing of uniform tablets.

2.2.3. By Moist Granulation Method

The mixed ingredients can be converted into granules by the following methods:

- Moist granulation method
- Dry granulation method
- Granulation by preliminary compression

2.2.3.1. Moist Granulation Method

This approach is the most popular one. Here, a sufficient amount of granulating agent is added to the evenly combined ingredients to create a cohesive mass. After that, the masses run through a No. 8 or 10 sieve. An excess of moisture is indicated if the mass adheres to the sieve's wire. In a hot air oven set to 60 degrees, the wet granules are spread out on trays and dried. To gather granules of uniform size, the dried granules are run through sieve No. 20.

2.2.4. Compression of Granules into Tablet

A tablet punching machine is used to compress the previously obtained dried granules into tablets. The necessary number of granules are loaded into the die, and they are compressed between the lower and upper punches to accomplish the compression. There are several different types of tablets making machines in use, such as the single punch tablet punching machine, multi punch tablet machine, rotary tablet, and dry coat tablet machine.

2.2.5. Evaluation Parameters

2.2.5.1. Angle of Repose

The angle of repose for each powdered poly herbal extract in the formulation was calculated using the fixed height method to determine the flow properties. A flat, smooth surface had a funnel fixed at a height of 2 cm, with a bottom diameter of 10 mm. A well-mixed sample weighing about 10 grams was gradually passed along the funnel's wall until the pile's tip formed and touched the funnel's bottom. A rough circle was drawn around the base of the pile, and the powder cone's radius was calculated [21].

Using the formula provided as equation, the angle of repose was computed by calculating the average radius.

Tan 0 = h / r

Where,
0= Angle of repose h= height of pile
r= average radius of powder pile

2.2.5.2. Bulk Density

The poly herbal powder mixture's bulk densities (BD) were ascertained by gradually adding 25 g of the sample mixture to a 100 ml graduated cylinder using a glass funnel. Records were kept of the sample's initial volume occupancy. Using the formula provided as eq., the bulk density was computed.

BD = Weight of the powder [g] / Volume occupied by the powder [ml]

2.2.5.3. Tapped Density [22]

The poly herbal powder mixture's tapped densities (TD) were ascertained by carefully adding 25 g of the sample mixture to a graduated 100 ml cylinder via a glass funnel. After tapping the cylinder from a height of two inches until a consistent volume was reached, the average value of each formulation was reported. Following tapping, the sample's final volume was measured, and the tapped density was computed using the formula provided in equation.

TD = Weight of the powder [g] / Tapped volume occupied by the powder [ml]

2.2.5.4. Compressibility

An effective empirical guide is provided by Carr's compressibility. By comparing the bulk density and tapped density of the poly herbal powder mixture, the compressibility of the mixture was determined. For every formulation, the percentage compressibility was computed using equation.

Carr’s Index = TD-BD / TD * 100

2.2.5.5. Hausner’s Ratio

It also demonstrates the densification of the herbal powder mixture, which was computed using the formula in eq. and may be caused by the feed hopper's vibration.
Hausner’s Ratio = TD / BD
Lower Hausner’s ratio - Better flowability Higher Hausner’s ratio - Poor flowability

2.2.5.6. Hardness

The Monsanto hardness tester is used to determine the tablets' hardness, which is then expressed in kg/cm2.

2.2.5.7. Thickness

Using a vernier calliper, the tablets' thickness is measured and recorded in millimeters (mm).

2.2.5.8. Weight Variation

20 tablets were taken from each bath, each of which was weighed separately, and the weight was recorded in order to examine the weight variations in the tablet formulation. After calculating the average weight of the tablets, it was further entered into the formula.

2.2.5.9. Friability

The tablets' friability is investigated using a Roche friability tester. Each batch of ten tablets is taken, and their initial weight is determined by weighing the tablets collectively. After that, the tablets are placed inside the device, and it is turned 100

times, or 25 revolutions per minute, for 4 minutes. The tablets are eventually taken out and dusted off. Friability is computed by weighing the de-dusted tablets and recording their final weight.

Percentage of Friability = [Initial Weight – Final weight] / Initial Weight * 100

2.2.5.10. Disintegration

900 milliliters of distilled water were added to the disintegration vessel, and six tablets from each batch were taken and loaded into the apparatus to begin the disintegration process. A constant 37 ± 2 °C is maintained. The frequency is adjusted between 28 and 32 cycles per minute.

2.2.5.11. Dissolution

The highly variable constituents in poly-herbal medicines make it challenging to oversee authority requirements for the dissolution testing of these medications. Poly-herbal medicinal products typically contain a combination of several different herbal constituents in their ingredients. The development of a dissolution method is far more involved than that of a defined single constituent.

2.2.5.12. Stability Studies

In order to quickly assess the physical, chemical, and physiological properties of the prepared formulation, an accelerated stability study was conducted in accordance with ICH guidelines for poly-herbal combinations. The optimized Poly Herbal Formulation was subjected to accelerated stability tests at different relative humidity and temperature levels.

3. Results and Discussion

Table 5. Observation of Pre-Formulation parameters.

Pre-Formulation Parameter	F1	F2	F3	Standard
Angle Of Repose	25.31	18.95	19.55	< 25
Bulk Density	.559	.607	.606	-
Tapped Density	.623	.665	.667	-
Compressibility	10.27	11.66	9.14	< 9
Hausner's Ratio	1.114	1.14	1.10	1.00-1.11

Table 6. Observation of Hardness.

Hardness				
Sr. No.	F1 [kg / cm2]	F2 [kg / cm2]	F3 [kg / cm2]	Standard Range
1	7.8	6.5	5.5	4-8 kg/cm^2
2	7.3	6.1	5.8	
3	6.9	6.8	6.0	
Average	7.3	6.4	5.7	

Table 7. Observation of Thickness.

Thickness			
Sr. No.	F1 [mm]	F2 [mm]	F3 [mm]
1	3.78	4.02	4.01
2	3.90	4.01	3.98
3	3.97	4.01	4.02
Average	3.83	4.02	4.01

Table 8. Observation of Weight Variation.

Weight Variation			
Sr. No.	Individual Weight F1	Individual Weight F2	Individual Weight F3
1	0.402	0.397	0.394
2	0.408	0.403	0.410
3	0.407	0.407	0.404
4	0.395	0.399	0.395
5	0.378	0.390	0.407
6	0.395	0.412	0.412
7	0.404	0.404	0.398
8	0.410	0.398	0.407
9	0.400	0.401	0.390
10	0.394	0.392	0.410
11	0.397	0.394	0.402
12	0.403	0.410	0.408
13	0.407	0.404	0.407
14	0.399	0.395	0.395
15	0.390	0.407	0.378
16	0.412	0.412	0.395
17	0.404	0.398	0.404
18	0.398	0.407	0.410
19	0.401	0.390	0.400
20	0.392	0.410	0.394
Average	0.398	0.400	0.401

Table 9. Observation of Friability.

Friability					
Formulation	Initial Weight	Final Weight	Difference	% Friability	Standard Range
F1	3.997	3.984	0.013	0.32%	Not more than 1 % [IP]
F2	4.006	3.990	0.016	0.39%	
F3	3.995	3.975	0.020	0.50%	

Table 10. Observation of Disintegration.

Disintegration				
Solvents	F1 (min)	F2 (min)	F3 (min)	Standard
Distil Water	35	32	28	Less than 60 min.
Buffer (PH 6.8)	27	24	22	
0.1 N HCl Acid	32	30	25	

Table 11. Observation of Stability testing parameters.

Stability Testing Parameters	Initial Parameters			After 30 Days			Standard
	F1	F2	F3	F1	F2	F3	
Hardness (kg/cm2)	7.3	6.4	5.7	7.2	6.4	5.7	4 - 8
Thickness (mm)	3.83	4.02	4.01	4.00	4.01	4.00	-

Weight Variation	0 no. of tablets were having weight variation of more than ± 5%			No Change			Above 250 mg tablet weight variation should be between + 5%
% Friability	0.32%	0.39%	0.50%	0.34%	0.40%	0.50%	Not more than 1 % [IP]
Disintegration							
Distil Water (min)	35	32	28	33	30	27	Less than 60 min
Buffer (PH 6.8) (min)	27	24	22	27	23	22	
0.1N HCl Acid (min)	32	30	25	30	29	23	

4. Conclusions and Future Scope

- In the above experiment we performed the preparation of poly herbal tablets (5 herbal plants) with bone-healing properties, and comparison of physical evaluation of three different tablet formulations with varying weight composition of the individual drugs and used excipients.
- Thus, from the observations of the evaluation parameters it is concluded that the F3 formulation tablet shows comparatively better in vitro results than F1 and F2 tablets.
- The reasons for that variations can be the appropriate use of different excipients in varying amounts, not only with that but also factors like individual powder characteristics, physical properties of drug powder are also liable to carry out the variations.
- Considering the references and researches done on the different herbs used, it can be said that the poly herbal tablets show their significance effect on bone healing properties along with their supporting actions of anti-inflammatory, anti-biotic, wound healing, Calcium supplement, providing immunity and strength, pain reliving – irritation, restrict blood loss etc.

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