



FORMULATION AND EVALUATION OF POLY HERBAL GRANULES AS DIETARY SUPPLEMENT

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ABSTRACT

The present research work is based on the formulation of poly herbal granules by incorporating the leaves extract of *Curcuma longa*, *Tinospora cordifolia* and *Withania somnifera*. The turmeric widely uses this plant for treatment of various diseases and disorder. The dried leaves powder of the plant was extracted and subjected to preliminary chemical tests. Then it was formulated and then evaluated for various parameters like angle of repose, bulk density, tab. Density, disintegration time and stability studies. The preliminary chemical studies show that the extract contains carbohydrate, alkaloids, flavonoid, glycoside and protein. The formulated poly herbal granules exhibited excellent flow properties which showed good angle of repose, bulk density and tapped density.

KEYWORDS: *Curcuma longa*, *Tinospora cordifolia*, *Withania somnifera*, poly herbal granules

INTRODUCTION

CURCUMA LONGA

Turmeric, derived from the rhizomes of *Curcuma longa*, is a perennial plant having short stem with large oblong leaves, and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in color. Accounting for about 78 percent of world turmeric production, India is the largest producer of turmeric.

Family: Zingiberaceae

Common name: Turmeric, haridra, haldi



Fig. 1: Curcuma longa

TINOSPORA CORDIFOLIA

Tinospora cordifolia is a large glabrous, perennial, deciduous, climbing shrub of weak and fleshy stem found throughout India.

Family: Menispermaceae

Synonyms:

Sanskrit: Guduchi, Gulvel

English: Heartleaf moonseed



Fig. 2: Tinospora cordifolia

WITHANIA SOMNIFERA

Withania somnifera is a small, woody shrub that grows about two feet in height. It can be found growing in Africa, the Mediterranean, and India.

Family: Solanaceae

Synonyms:

English: Winter cherry

Hindi: Ashwangadha

Sanskrit: Turangi-gandha



Fig. 3: Withania somnifera

MATERIAL AND METHODS

Plant Material:

Collection and drying:

The botanical staple consisting of three medicinal plants *Curcuma longa* (rhizome), *Withania somnifera* (roots), *Tinospora cordifolia* (leaves), were procured from the authenticated local market of Ahmednagar district.

Dried material was coarsely powdered in grinder and powder was used for extraction.

Authentication:

The identities of the plants were confirmed by the Botany department of sangamner college of arts, science & commerce, Tal: Sangamner dist.: Ahmednagar.

Extraction of plant material:

Method: Soxhlet extraction

Solvents: Using H₂O and alcohol.

The collected plant parts were crushed to powder.

The powder was extracted with 7:3 hydro alcoholic mixtures, at 60°C temperature, for 6 hrs, in a 500ml round bottom flask. After 6 hrs. of extraction, round bottom flask was cooled to temperature and the extract were filtered and picked up. Decoction was prepared by evaporating the extract to at least one third of its volume.

Decoction was poured onto a glass tray and dried at 100°C.

Dried extract was pulverized and stored in a desiccator.



Fig. 4: Extraction process

Preliminary phytochemical screening for various extracts: (Khandelwal, 2005)

Test for carbohydrates:

Molisch test (General test):

Two ml of extract solution was added with few drops of 1% methanolic α -naphthol solutions in a test tube and 2ml of concentrated sulphuric acid was added carefully along the side of the test tube. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

Test for reducing sugar:

Benedict's test:

Mix equal volume of Benedict's reagent and extract solution in the test tube. Heat in a boiling water bath for 5 min. solution appears green, yellow or red depending on amount of reducing sugar present.

Fehling's test:

Five ml of extract solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled. Development of reddish brown precipitate indicates the presence of reducing sugars.

Test for Alkaloids:

Evaporate all extracts separately. To residue, add dilute HCL. Shake well and filter. Use filtered solution for test.

Dragendroff's test:

2-3 ml test solution and 0.1 ml Dragendroff's reagent was added in test tube.

Formation of orange brown precipitate indicates the presence of alkaloids

Mayer's test:

2-3 ml test solution and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

Hager's test:

2-3 ml test solution and 0.1 ml of Hager's reagent. Formation of yellowish precipitate indicates the presence of alkaloids.

Wagner's test:

2-3ml filtrate with few drops Wagner's reagent gives reddish brown ppt.

Acid test:

Test solution treated with tannic acid solution gives buff colored precipitate.

Test for Flavonoids:**Shinoda test:**

To dry powder or extract, add 5 ml 95% ethanol/t-butyl alcohol, few drops conc.HCL and 0.5g magnesium turnings. Orange, pink, red to purple color appears (flavonols, dihydro derivatives and xanthenes).

Sulphuric acid test:

On addition of sulphuric acid (66% or 80%) flavones and flavonols dissolve into it and deep yellow solutions. Chalcones and aurones give red or red-bluish solutions. Flavones give orange to red colors formed.

Addition of accelerating amount of sodium hydroxide to the residue shows coloration, which decolorizes after addition of acid.

Heat test solution with zinc and HCL, pink to red color is observed.

Test for Tannins:

- To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents.
- 5% FeCl_3 solution: Deep blue-black color.
- Lead acetate solution: White ppt.
- Gelatin solution: White ppt.
- Bromine water: Decolouration of bromine water.
- Acetic acid solution: Red color solution.
- Potassium dichromate: Red ppt.
- Dilute Iodine solution: Transient red color.

Detection of Tannins:

- Solvent system used:
- Toluene: Acetone: Ethyl acetate (3:1:2)
- Ethyl acetate: Formic acid: Acetic acid: water (100:11:11:26)

- Spray reagents:
- 5% FeCl₃ in 0.1N HCL. The developed TLC plate was sprayed with reagent, heated at 100°C for 5-10 minutes.
- Color observed: bluish black spots.

Preparation of Poly herbal granules:

Selection of excipients:

Starch was chosen as disintegrant, calcium phosphate dibasic as bulking agent, magnesium stearate as antiadherent, and methyl and propyl parabens as preservatives. To mask the acute bitter taste, sucralose was used as sweetener, citric acid as taste masker.

Formulation of poly herbal granules:

Granules were prepared by using wet granulation technique. Extract (powder) and citric acid were mixed in a mortar to which sucralose were added. This was followed by subsequent addition of starch, calcium phosphate dibasic and therefore the parabens. Sufficient quantity of distilled water was added to form a lumpy mass which was then passed through sieve no. 22 to form granules. Granules were dried within the oven. Magnesium stearate was added at the end for lubrication.

Table 4. Formulation table

Sr.no	Ingredients	Quantity(mg)	Category
1	Extract	125	----
2	Starch	150	Disintegrant
3	Magnesium stearate	2.5	Antiadherent
4	Calcium phosphate dibasic	250	Bulking agent
5	Pearlitol	312.5	Bulking agent
6	Citric acid	125	Taste masker
7	Methyl parabens	2 ml	Preservative
8	Propyl parabens	0.5 ml	Preservative
9	Orange Flavor	Qs	Flavoring agent
10	Sucralose	Qs	Sweetening agent
11	Color	Qs	Coloring agent

Evaluation of poly herbal granules: (Lachman- Liberman 1999, USP 2007)

Angle of Repose:

The angle of repose is that the angle formed by the horizontal base of the bench surface and the edge of a cone-like pile of granules. After the cone from 5 g of sample was built, height of the granules forming the cone (h) and therefore the radius

(r) of the base were measured.

The angle of repose (θ) was calculated as follows: $\theta = \tan^{-1} (h/r)$

Results were only considered valid when a symmetrical cone of powder was formed. The funnel method was used to perform the test.

Loss on drying:

This test was performed by drying a weighed quantity of the product in the oven at 105°C until constant weight was obtained.

Bulk density:

- It is that the ratio of total mass of powder to the bulk volume of powder.
- $D_b = m / V_O$
- Where, m: Mass of the blend
- V_O : Untapped Volume
- A graduated glass cylinder was used to perform the test

Tapped Density:

Tapped density is the ratio of mass of powder to the tapped volume. Tapped volume is the volume occupied by the same mass of the powder after a standard tapping of a measure.

- $D_t = m / V_i$
- Where, m: Mass of the blend.
- V_i : Tapped Volume
- Graduated glass cylinder was used for the test which was subjected to 50 tapping and the volume was noted.

Disintegration time:

The test was performed using a beaker containing simulated saliva fluid maintained at 37 °C for evaluating fast disintegration. The formulation was added to it and the disintegration time was noted.

Stability Studies:

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile. The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of 25°C/60% RH, 30°C/60% for 3 months. After the completion of three month the samples were analyzed visually for any color changes due to physical and chemical interaction within excipients and with the drug. (ICH guidelines 1993).

Screening of Polyherbal granules for antioxidant activity:

Formulated poly herbal granules were evaluated for its antioxidant action using DPPH radical scavenging method:

DPPH assay (2, 2-Diphenyl 1-1-Picrylhydrazyl):

The free radical scavenging activity of extract was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (50mg/ml) in methanol was used as reference standard.

Principle:

2,2 Diphenyl 1,1- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

Procedure:

Evaluation of antioxidant activity by DPPH radical scavenging method Free radical scavenging activity of extract were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (25, 50, 100 µg/ml). Here, only those extracts are used which are Solubilise in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30min. then, absorbance was measured at 517 nm. By using spectrophotometer (UV- VIS Shimadzu). Reference standard compound being used was ascorbic acid. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using formula. Lower absorbance of the reaction mixture indicated higher free radical activity.

The percent DPPH scavenging effect was calculated by using following equation:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100.$$

Where, A₀ was the Absorbance of control reaction

A₁ was the Absorbance in presence of test or standard sample.

EXPERIMENTAL RESULTS

Phytochemical screening:

Table 5. Preliminary Phytochemical Screening

Type of phytoconstituents	Inference
Carbohydrates	+
Alkaloids	+
Tannins	+
Flavonoids	+

Excipients profile:

Starch takes up water from the body fluids which cause it to swell and thereby leading to disintegration of the granules. Calcium phosphate dibasic used as bulking agent. Pearlitol also aids in faster disintegration and acts as a non-calorific sweetening agent. Magnesium stearate helps to prevent attrition between the granules and formation of fines. Methyl and propyl parabens are nontoxic, non-irritating and are used in combination to prevent decomposition of the formulation. Citric acid helps to stimulate salivary secretions and hence leading to disintegration of the granules in the oral cavity, thereby obviating the need to consume water along with the formulation.

Sucralose and the flavoring agent help to mask the bitter taste of extract. 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.

Evaluation of Polyherbal granules:

The values of 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. The disintegration test implies that the granules can disintegrate within 15 sec, thereby leading to quicker absorption and onset of action of the drug as compared to that in its other dosage form.

Stability study discussion:

After the three months of completion the samples were analyzed visually for any color changes and hence no color changes were observed due to physical and chemical interaction within excipients and with the drug.

Table 6. Evaluation of Polyherbal granules

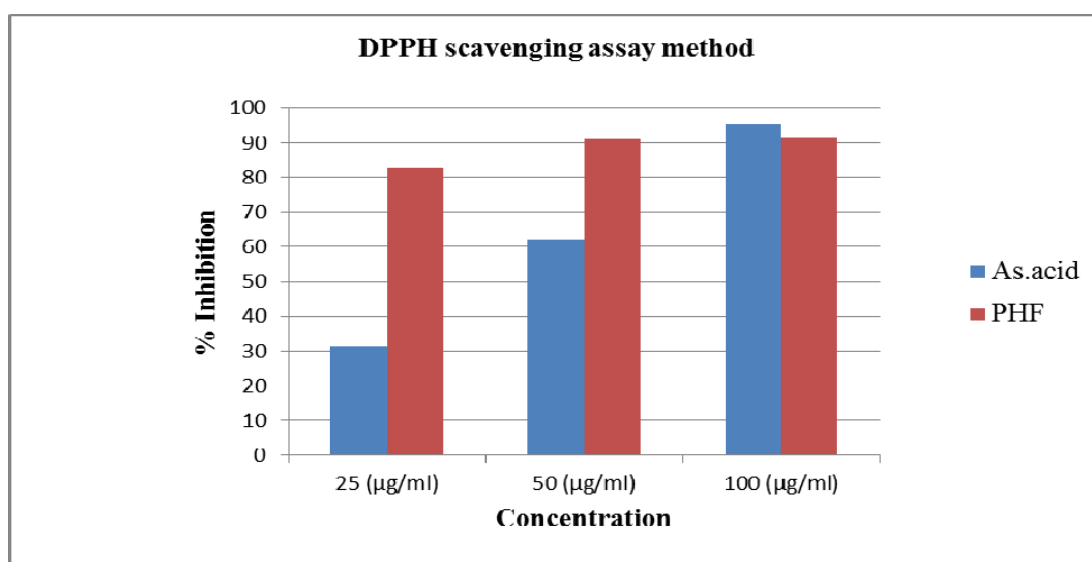
Parameters	Results
Angle of repose	26.38
Loss on drying	0.355
Bulk density	0.498
Tapped density	1.20
Disintegration time	Within 15 sec
Stability study	Stable

Screening of polyherbal granules for antioxidant activity:**Total antioxidant percent:**

Absorbance of with standard ascorbic acid at 517 nm by UV visible spectrophotometer (dpsh scavenging assay method)

Table 7. Total antioxidant percent

Sr. no	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Polyherbal formulation (%inhibition)
1	25	31.22 %	82.56 %
2	50	61.95 %	90.97 %
3	100	95.12 %	91.34 %



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