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RESEARCH ARTICLE OPEN ACCESS

Development and Assessment of Polyherbal Granules As A Diatary Supplement

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ABSTRACT
The purpose of the current study is to create utilize-herbal granules using the leaves of Curcuma longa, Tinospora cordifolia, and Withania somnifera. This plant is used extensively in the treatment of numerous ailments and disorders by the turmeric. The plant's dried leaf powder was taken out and put through some initial chemical testing. It was then developed, assessed, and tested for a number of factors including angle of repose, bulk density, tab density, disintegration time, and stability investigations. According to the preliminary chemical analyses, the extract contains protein, alkaloids, flavonoids, glycosides, and carbohydrates. The created polyherbal granules displayed great flow characteristics, including a favourable angle of repose, bulk density, and tapped density. There was minimal research done on dietary supplements up until quite recently, therefore nothing was known about them. However, during the past 20 years, the use of supplements has drastically increased, and this has sparked consumer interest. Any vitamin, mineral, auxiliary chemical, biological, or seasoning product added to the diet to improve human health is referred to as a dietary supplement. Dietary supplements should avoid making claims that they treat cardiovascular disease or lessen pain, for example. For years, nutritionists and health experts have claimed that a typical, balanced meal should provide all of the essential nutrients that an individual's body need each day. Over forty nutrients are covered in today's dietary advice from health and nutrition organizations, which is broken down into six groups: carbs, fats, proteins, vitamins, minerals, and water.

Keywords: Polyherbal granules, Curcuma longa, Tinospora cordifolia, and Withania somnifera.

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I. Introduction

Dietary Supplements:

Until comparatively recently, there was restricted research project on dietary supplements so very little was celebrated concerning them. However, the prevalence of supplement use has redoubled dramatically over the past twenty years, and that they have become a matter of shopper interest. Dietary supplement is outlined as any vitamin, mineral, accessorial chemical substances, biology or seasoner merchandise that's added to the diet to boost human health. Scientists and health professionals agree that dietary supplements are underneath sure conditions useful to human health, but shouldn't replace complete and balanced daily meals of foods that are necessary for a healthful diet. The foremost authoritative national agency U.S. Food and Drug Administration (FDA) stressed that in contrast to medicine, dietary supplements are not supposed to treat, diagnose, prevent, or cure diseases. Dietary supplements should not create claims, like reduces pain or treats cardiovascular disease etc. Nutritionists and health professionals argued for years that folks will get the most important food needs that their body desires day after day from a standard, balanced and regular daily diet. Today's dietary tips from health and nutrition agencies cover over forty nutrients that are unit divided into six categories: carbohydrates, fats, proteins, vitamins, minerals and water. (Price S.2005)

Daily nutrient recommendations area unit together called dietary reference intakes (DRIs). A healthy diet is one that favors real contemporary whole foods that have been sustaining people throughout the millenniums. Whole foods supplies provide the required vitamins, minerals, protein, carbohydrates, fats, and fiber that are essential to good health. In contrast, commercially ready and quick foods are usually lacking nutrients and contain inordinate amounts of sugar, salt, saturated and trans-fats, all of which are associated with the event of diseases. A diet may be mixture of food from the various food teams (vegetables, legumes, fruits, grains, protein foods, meat, and dairy). Variety

involves consumption completely different foods from allthe food teams that help to confirm that you receive all the nutrients necessary for a healthy diet. (Katz DL, Mellor S.2014) In the last decade national medical authorities, health professionals and nutritionists in developed countries became alert to the widespread and rapid increase of dietary supplements (DS) and excessive consumption by a wide range of thepopulation. The global market is flooded with a variety of dietary supplements that have false therapeutic claims (difficult to test experimentally) and products which can be imported and distributed through the internet advertisements. Like drugs, dietary supplements have risks and facet effects. But sellers aren't required to do research studies in people to prove that dietary supplements are safe. In contrast to medicine, DS are largely self-prescribed with no input from informed medical sources like doctors, nurses, or pharmacists. Medical authorities agree that there's heaps of wrong data within the supplements market.

Polyherbal granules:

Herbal medication is that the oldest type of health care noted to world being associate integral half within the development of the fationable day civilization. In herbal medicine plant based formulation is used to alleviate diseases. However, the foremost challenges faced by these formulations arise attributable to their lack of complete analysis. Granules is used as a unique approach for mistreatment flavoring drug as they offer advantages such as faster disintegration and dissolution as compared to tablets and capsules, eatables as compared to syrups and decoctions, bigger acceptableness in patients thanks to lesser risk of choking, time and cost required for their manufacture is lesser as compared to tablets The development of poly herbal formulation is challenging task because of the large number of varied chemical compounds presentin the different medicinal plants, hence the entire herbal drug or preparation is regarded a active Drug substance.

Pharmacological activities of *Tinospora cordifolia:*
Antioxidant Activity:
The toxicity induced by free radicals is definitely reduced by the extract of Tinospora cordifolia. The plant extract inhibits the lipid per oxidation, generation of superoxide and hydroxyl radicals (in-vitro). The toxic side effects caused by cyclophosphamide in mice as shown by elevated lipid peroxides in serum and liver, as well as alkaline phosphatase and glutamine pyruvate transaminase are also partially reduced by plant extract.

Wound Healing Activity:
The wound healing activity of *Tinospora cordifolia* has been reported in albino rats. The result concluded that the plant may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that hastens the process of wound healing.

Anti-Inflammatory Activity:
The stem aqueous extract of *Tinospora cordifolia* exerted a significant anti- inflammatory effect on cotton pellet granuloma and formalin induced arthritis models. Its effect was comparable with Indomethacin. The plant produced significant anti-inflammatory effect in both acute and sub-acute models of inflammation.

Anti-malarial Activity
The effect of aqueous extract of *Tinospora cordifolia* along with chloroquine in the treatment of three cases of hyper reactive malarious splenomegaly (HMS) was studied. The plant extract (500 mg/kg b.w.) added to chloroquine (CQ) base (300 mg/kg b.w.) was administered weekly. The results showed regression of spleen by 37-50% after six Weeks and 45- 69% after six months. Decrease in IgM and increase in Hb also were observed.

Pharmacological activities of *Withania somnifera:*
Antioxidant activity:
The brain and nervous system are relatively more vulnerable to radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. Free radical damage of nerve tissue could also be involved in normal aging and Neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), are tested for antioxidant activity using the main free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) within the rat brain frontal area and striatum.

Anxiety and depression:
Anxiolytic and antidepressant actions of the bioactive WSG, isolated from WSroots, in rats were assessed. WSG was administered orally once daily for 5 days and the results were compared by those elicited by the benzodiazepine lorazepam for anxiolytic activity, and by the tricyclic antidepressant,imipramine.

Antibacterial effect:
Both aqueous as also as alcoholic extracts of the plant (root also as leaves) were found to possess strong antibacterial activity against a variety of bacteria, as

revealed by in vitro Agar Well Diffusion Method. The methanolic extract was further sub fractionated using various solvents and therefore the butanolic sub-fraction was possessed maximum inhibitory activity against a spectrum of bacteria including Salmonella development of dependence to opiate as assessed by naloxone precipitation withdrawal on day 10 of testing. The studies revealed that the chronic administration of the WS didn't exhibit any dependence-liability of its own, even upon an abrupt cessation. These findings may have clinical implications without producing tolerance and withdrawal effects on long-term use.

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.

Figure 1: *Curcuma longa*

Tinospora cordifolia:
Tinospora cordifolia is a large glabrous, perennial, deciduous, climbing shrub ofweak and fleshly stem found throughout India.

Figure 2: *Tinospora cordifolia*

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

Figure no 3: *Withania somnifera*

II. 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.
Extraction of plant material:
Method: Soxhelet 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 600C 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 desicator.

Figure No 4: Extraction process

Preliminary phytochemical screening for various extracts:
Test for carbohydrates:

Molisch test (General test):
Two ml of extract solution was added with few drops of 15 methanolic alpha- 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 precipitated 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-bluishsolutions. 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℃ 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 antiadherant, 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.1 formulation table

Sr.no	Ingredients	Quantity(mg)	Category
1	Extract	125	-
2	Starch	150	Disintegrant
3	Magnesium stearate	2.5	Antiadherant
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: θ = 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.
- Db = m / VO
- Where, m: Mass of the blend
- VO: 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.

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

Disintegration time:

The test was performed using a beaker containing simulated saliva fluid maintained at 37 ^{0}C for evaluating fast disintegration. The formulation was added to itand 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 atspecified 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 guidelines1993).

Screening of Polyherbal granules for antioxidant activity:

Formulated poly herbal granules were evaluated for

its antioxidant action usingDPPH radical scavenging method:

DPPH assay (2, 2-Dipheny l-1-Picrylhydrazyl):

The free radical scavenging activity of extract was determined by using DPPHassay. 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 withred 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:

Percent inhibition = A0 - A 1 / A0 × 100.

Where, A0 was the Absorbance of control reaction A1 was the Absorbance in presence of test or standard sample.

III. Experimental Results

Phytochemical screening:

Table no 2: 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 withthe 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 makesthe 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 no 3: 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 polyherbalgranules for antioxidant activity:

Total antioxidant percent:

Absorbance of with standard ascorbic acid at 517 nm by UV visiblespectrophotometer (dpph scavenging assay method)

Table no 4: 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 %

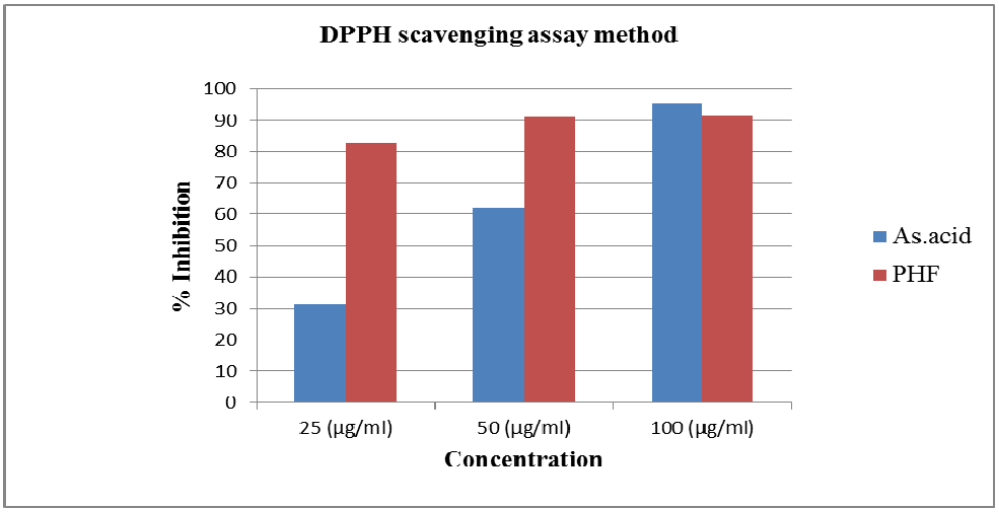

Fig. No. DPPH scavenging method

IV. Conclusion

Herbs in the form of dietary supplement play major role in the treatment than allopathic medicine because of less side effect and easy availability. The formulation was prepared with excipients and flavoring gents to improve patient compliance. Citric acid which also acts as sialagogue was used, thereby obviating theneed of consuming water while administering the formulation. The granules apart from showing excellent flow properties disintegrate within 15 seconds in oral cavity. As wet granulation technique is used, the process is cheaper and less time consuming as compared to production of tablets, capsules and

syrups.The result obtained from above study indicates the presence of flavonoids, alkaloids, carbohydrates & tannins in the Polyherbal formulation. The antioxidant screening done by using DPPH method showed that the free radical scavenging effect of PHF at concentration 100µg/ml (i.e. 91.34%) showed maximum % inhibition of free radicals. The effects showed at concentration 25 and 50 µg/ml were found to be more antioxidant potential than reference standard drug. From theabove study we can conclude that poly herbal formulation possesses promising Antioxidant activity which can be considered as base for further pharmacological evaluation. Hence, this formulation can serve as an ideal candidate for commercialization onlarge scale and an inexpensive therapy as compared to currently. The present study was done on *Curcuma long* (rhizome), *Tinospora cordifolia* (leaves), *Withania somnifera* (roots).The powder of above plant parts were extracted by solvents. The poly herbal granules formulated from the above extract will be beneficial for human being as dietary supplement.

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