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Formulation, Evaluation Of Polyherbal Dosage Form, And Study Of Its Antioxidant Activity

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ABSTRACT

Objective: The study was designed as formulation, standardization, and evaluation of polyherbal tablets prepared for the management of antioxidants. To overcome the problem of dyspepsia in geriatric patients by the use of polyherbal tablets and powder formulations.

Methods: Powder and tablets were prepared using aqueous root extract powder of the selected plant viz. Hibiscus rosa sinesis, and sorghum bicolor with the help of super disintegrant addition technique using crospovidone, sodium starch glycolate, and croscarmellose sodium in different percentages. Evaluation assessments such as the substantial test, weight variation, hardness, friability, content uniformity, disintegration, in vitro dispersion, stability study were carried out.

Results: Micromeritics of extracts powder were determined for all formulations, which signified good flow properties. A substantial examination was established, which comply with official requirements for uniformity test, and the drug content was close to 90% in all formulations. Disintegration time was observed for all formulations in which the polyherbal formulation-3 (PHF-3) showed 1.10±0.10 min; during in vitro dispersion time, all formulations showed appropriate dispersion in which the PHF-3 captivating 2.00±0.45 min only.

Conclusion: The PHF-3 showed satisfactory disintegration and in vitro dispersion time due to crospovidone and was reported as the best formulation. The stability study and antioxidant property validate the PHF may represent a new easily swallow dispersible tablet that may enhance drug permeability and advance bioavailability for oxidant needed patients.

Keywords: Polyherbal dispersible tablet, Micromeritics, Dispersion time, Superdisintegrants, Crospovidone.

INTRODUCTION

Ayurveda is one of the traditional medicinal systems with an established history of many centuries. Furthermore known as Ayurvedic medicine, this ancient Vedic knowledge is considered to be one of the oldest healing sciences and has survived until the present generation over many centuries of tradition. Originated in India thousands of years ago, Ayurveda is known as the 'mother of all healing'. Etymologically speaking it is the combination of Sanskrit words Ayur (life) and Veda (science or

knowledge), which means “the science of life”, focusing on bringing harmony and balance in all areas of life including mind, body and spirit. In Ayurveda, panchamahabhutas are the five elements: Vayu (air), Teja (fire), APA (water), Prithvi (earth), and akasha (void) are believed to build up the living microcosm (human beings) and the macrocosm (external universe). All these present the constitution or Prakriti of an individual which determines the physical as well as mental characteristics of humans. The concept is the balance between these three fundamental doshas, whereas imbalance causes diseases. Based on panchamahabhutas and tridosha, the Prakriti of an individual is determined and a typical treatment plan can be prescribed according to their unique constitution. (**Kshirsagar M et al., 2011**) The beliefs of Ayurveda are to prevent unnecessary suffering and live a long healthy life. Unlike the allopathic medicines which use mainly synthetic chemicals designed for specific target receptors and primarily give symptomatic relief, Ayurveda involves the use of natural means such as diet, herbs, spices, minerals, exercise, meditation, yoga, mental hygiene, sounds, smells and mechano-procedures to eliminate the root cause of the disease by restoring balance, at the same time create a healthy lifestyle to prevent the reoccurrence of the imbalance. Ayurveda is said to be holistic as it aims to integrate and balance body, mind, and spirit to prevent illness and promote wellness, longevity, vitality, and happiness. **(Dahanukar SA 1989**) In living systems, oxidation is a basic part of the normal metabolic process, in which reactive oxygen species and many free radicals are generated. Continuous production of free radicals may cause the structure and function of cell constituents and membranes and can result in neurologic and other disorders like cancer, inflammatory disease, diabetes, asthma, cardiovascular, neurodegenerative diseases, and premature aging. Hence, the prevention of the above condition needs the presence of antioxidants or free radical scavenging molecules. There is plenty of antioxidant substance present in plants and the free radical scavenging molecules present in them are in the form of phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous medicine. So to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above disease conditions.

MATERIALS AND METHODS

Preparation of dried aqueous extract

100 g of the polyherbal mixture was taken in 800ml water and a decoction was prepared as per Sarangdhar Samhita (Anonymous 2007). A decoction was filtered through muslin cloth to obtain 200 ml of aqueous extract, concentrated under vacuum using rotatory evaporator (Superfit, Inst. IDN code: PC50) at 800 C for 48 hours to remove the water content and obtain the extract in dry solid form. (**Gopalasatheeskumar K et al., 2017**) The extract was then standardized as per WHO guidelines of quality standardization (**Aslan M et al., 2010**) and Ayurvedic Pharmacopoeia of India (API) (**Kopleman SH et al., 2001).**

Preparation of Polyherbal tablet

The plant materials (Hibiscus rosa sinesis and Sorghum bicolor) were separated from earthy and other foreign material; shade dried and powdered using a mill. Polyherbal mixture was prepared using the above-mentioned three ingredients in an equal ratio (**Satyanarayan S. et al., 1998**).

The polyherbal formulation, Dosage form was formulated by filling the polyherbal powder mixture, standardized as per WHO guidelines of quality standardization. Characterization of Polyherbal tablet and powder. The developed polyherbal tablet was characterized for the following physicochemical parameters.

Characterization of powder

The biologically potent polyherbal extract powder stands for varied physical properties and micrometric properties. Extract powdered are heterogeneous because it was composed of individual particles of different sizes and shapes randomly interspersed with air spaces and becomes more complicated with polyherbal [16, 17]. Measurements were carried out in triplicate for each formulation and presented as the average±standard deviation (SD).

Angle of repose

The flow properties of thoroughly mixed all polyherbal extract powder in the formulation were determined by calculating the angle of repose by the fixed height method. A funnel with 10 mm in diameter of the bottom was fixed at the height of 2 cm over the plain and smooth surface. About 10 gm of a thoroughly mixed sample was slowly passed beside the wall of the funnel until the tip of the pile formed and touched the bottom of the funnel. A rough circle drowned around the pile base, and the radius of the powder cone was measured [18]. The angle of repose was calculated by the average radius using the following formula given as eq. 1.

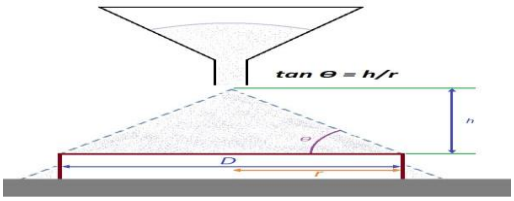


Figure no. 1: Angle of Repose

Where, θ = angle of repose h = height of the pile r = average radius of the powder cone.

Bulk density

The bulk densities (BD) of the polyherbal powder mixture were determined by pouring gently 25 gm of sample mixture through a glass funnel into a 100 ml graduated cylinder. The initial volumes occupied by the sample were recorded. The bulk density was calculated by using the following formula given as eq. 2.

Tapped density

The tapped densities (TD) of the polyherbal powder mixture were determined by pouring gently 25 gm of sample mixture through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from the height of 2 inches until a constant volume was obtained and then the average value of all formulations was reported. The final volume occupied by the sample after tapping was recorded and tapped density was calculated by using the formula given as eq. 3.

$$\text{Bulk density}(\rho_b) = \frac{\text{Weight of microcapsules(g)(M)}}{\text{Bulk volume(ml)}(V_b)} \quad (2)$$

$$\text{True/Tapped density}(\rho_t) = \frac{\text{Weight of microcapsules(g)(M)}}{\text{Tapped volume(ml)}(V_t)} \quad (3)$$

Figure no. 2: Bulk and Tapped density

Compressibility

Carr's compressibility gives a useful empirical guide. The compressibility of the polyherbal powder mixture was calculated by comparing the bulk density and tapped density.

Hausner's ratio

It also shows densification of herbal powder mixture which may result from the vibration of the feed hopper, which was calculated by using the formula.

$$Compressibility\ Index = 100 \times \left(\frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \right)$$

$$Hausner\ \ Ratio = \left(\frac{\rho_{tapped}}{\rho_{bulk}} \right)$$

Figure no. 3: Compressibility and Hausner Ratio

Preparation of polyherbal dispersible tablet

Polyherbal dispersible tablets were prepared separately through geometrical dilution mixture with direct compression method (Punching machines, Cadmach CMS-15 No. H/513/11-12) by using different ingredients, disintegrating agents, talc, etc. as the composition of these formulations and denoted by PHF-1 to PHF-9. All the ingredients were passed through a mesh sieve no. 120 and then mixed each other by using the geometrical dilution method to maintain uniformity. Powder mixtures possess good flow properties and good packing ability; therefore, the mixtures were directly compressed [20].

Evaluation of polyherbal Tablet

Weight variation test

Test for uniformity of weight was performed as per Indian Pharmacopeia (IP), 2007 (**Chawla R, Kumar V., et al., 2013**). Randomly selected 20 Tablets were weighed (individually and together) in a single pan balance. The average weight, variation in the individual Tablet, and the standard deviation were calculated. IP limit for weight variation in the case of tablets weighing more than 300 mg is ± 5%. Moisture

analysis. The Tablet was weighed, kept in an oven at 105^{o}C, and equilibrated. It was reweighed again till three constant readings using moisture balance and moisture content was measured gravimetrically (**Chui SH et al., 2013**).

Drug content

Test for drug content was carried out as per IP (**Chawla R, Kumar V., et al., 2013**). Twenty tablets were taken and emptied their content in a mortar and pestle. In a volumetric flask, 250 mg of powder was taken and diluted with phosphate buffer (pH 6.8). The absorbance of the solution was measured at 213 nm (λ max of the formulation calculated by scanning the different concentrations of it in phosphate buffer pH 6.8 within a range of 200 to 400 nm (**Savrikar S.S. et al., 2010**) using UV/visible spectrophotometer (Shimadzu 1601 UV–VIS Spectrophotometer, Japan). The amount of drug present in the individual capsule was then estimated using the standard calibration curve.

Disintegration time

From each formulation 6 polyherbal dispersible tablets were randomly selected to determine the disintegration time. The acidic buffer (pH 1.2) was used as disintegration medium and the temperature was maintained at 37±0.5 °C. The average disintegration time of six tablets was noted down for calculation [22].

Dispersion time

In vitro dispersion time of polyherbal dispersible tablet was measured by dropping the tablet in a beaker containing 100 ml of normal water. Two tablets from each formulation were randomly selected and in vitro dispersion time was determined. A smooth dispersion was produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm [23, 24]

Stability studies

An accelerated stability study was carried out as per ICH guidelines for polyherbal combination to check the physical, chemical, and physiological properties of prepared formulation in a short period. The optimized PHF was subjected to accelerated stability studies at three different conditions of temperature and relative humidity i.e., 25 °C/60% RH, 30 °C/65% RH, and 40 °C/75% RH for 3 mo [26].

Dissolution study

The dissolution profile of capsule formulation containing polyherbal extract was determined according to USP type-I dissolution tester apparatus (rotating basket) (Thermonix Campbell, Inst. IDN code: PC42). An accurately weighed amount of capsule was placed in USP dissolution basket rotated at 100 ± 5 rpm using phosphate-buffered saline (PBS) (pH= 6.8) as a dissolution medium and temperature was adjusted to 37 0C ± 0.5. 3 ml aliquot of sample was withdrawn at regular time intervals (0, 30, 45, 60, 90, and 120 min) diluted and assayed spectrophotometrically at 213 nm. Meanwhile, an equal volume of PBS was added to maintain the constant volume. The cumulative % release was calculated for the formulation from the previously constructed calibration curve.

In vitro Screening of Antioxidant Activity

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of the extract was measured by the Blois method. 0.3mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 1ml of various concentrations of the sample (12.5, 25, 50, 100, 200, and 400µg/ml) and the reference compound (5, 10, 15, 20, 25, and 30µg/ml), shaken vigorously, left to stand in the dark at room temperature for 30min and then absorbance was measured at 517nm against a blank. The reference compound used here was Ascorbic Acid. A control reaction was carried out without the test sample. All the tests were performed in triplicate to get the mean values. (**Saravanan J. et al., 2018**) The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. The antiradical activity was expressed as percentage inhibition (I %) and calculated using the following equation:

Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100

Different sample concentrations were used to obtain calibration curves and to calculate the IC50 values. (IC50 - concentration required to obtain a 50% radical scavenging activity).

RESULTS AND DISCUSSION

Characterization of powder

The basic characterization of powder and micrometric properties of formulations containing Polyherbal aqueous leaf extracts powder used mentioned in table 1.

Table 1: Micromeritic parameters of polyherbal aqueous root extracts powder

Formulation Code	Bulk density (gm/ml)	Tapped density (gm/ml)	% Compressibility	Hausner Ratio	Angle of Repose (°)
PHF-1	0.38±0.05	0.55±0.06	20.00	1.24±0.10	32.12±1.82
PHF-2	0.42±0.04	0.53±0.08	28.57	1.25±0.09	30.20±2.01
PHF-3	0.35±0.03	0.49±0.07	21.82	1.40±0.13	24.35±1.00
PHF-4	0.40±0.06	0.50±0.06	28.30	1.31±0.11	26.24±1.32
PHF-5	0.45±0.04	0.56±0.08	19.64	1.39±0.14	26.48±1.02
PHF-6	0.38±0.07	0.50±0.07	24.00	1.35±0.14	28.22±1.56
PHF-7	0.43±0.06	0.52±0.05	17.31	1.36±0.12	28.32±1.37
PHF-8	0.40±0.04	0.57±0.08	25.93	1.21±0.09	27.20±1.65
PHF-9	0.42±0.05	0.54±0.06	26.32	1.32±0.08	26.50±1.22

Formulation and characterization of tablets

Polyherbal dispersible tablets were compressed each of 550 mg weight on a 10-station Mini Press-I rotary tablet compression machine fitted with 12 mm punches size. None tablet manufacturing defects like capping, lamination, and chipping were observed (table 2)

Table 2: Composition of Polyherbal formulation for dispersible tablets

Ingredients (mg/tab)	PHF-1	PHF-2	PHF-3	PHF-4	PHF-5	PHF-6	PHF-7	PHF-8	PHF-9
Aqueous extracts	25X10 =	25X10 =	25X10 =	25X10 =	25X10 =	25X10 =	25X10 =	25X10 =	25X10 =
Powder (3 plants)	250	250	250	250	250	250	250	250	250
β-cyclodextrin	200	200	200	200	200	200	200	200	200
Microcrystalline cellulose	65	60	55	50	65	60	55	50	50
Sodium Saccharin	10	10	10	10	10	10	10	10	10
Mg. Stearate	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5
Total Weight	550	550	550	550	550	550	550	550	550

Herbal Tablet Formulation

Herbal Powder Formulation

All the prepared formulations were subjected to evaluation of characteristic parameters like size, shape, color, and appearance. The prepared polyherbal dispersible tablets were non-sticky and looked high-quality. The diameter and thickness of each formulated tablet were performed with 20 tablets by using a digital vernier scale during the physical study because it permits accurate measurements and provides exact information about variations between tablets of each formulation (table 3). The visualized color and shape of all tablets were observed directly by naked eyes (fig. 4).

Table 3: Physical description of polyherbal dispersible tablets

S. No.	Parameter	Result
1	Color	Yellowish-Brown
2	Shape	Round, Biconvex
3	Odor	Characteristic odor
4	Taste	Pleasant taste

Table 4: Physical properties of polyherbal tablets

Formulation code	Average weight (mg	Weight variation (%	Content uniformity (%	Hardness (kg/cm2)	Friability (%)
PHF-1	560.80±10.2	1.93	099.85	2.94±0.13	0.90
PHF-2	565.31±8.11	2.71	103.10	3.00±0.12	0.86
PHF-3	563.28±7.78	2.36	105.05	2.99±0.14	0.79
PHF-4	562.40±8.01	2.20	101.25	2.91±0.09	0.82
PHF-5	563.15±9.38	2.34	102.38	2.98±0.13	0.90
PHF-6	564.13±8.78	2.50	099.68	2.97±0.11	0.88
PHF-7	563.18±8.07	2.34	102.00	3.02±0.18	0.78
PHF-8	558.34±7.68	1.22	098.96	2.96±0.16	0.85
PHF-9	563.63±7.40	2.42	101.80	2.95±0.12	0.80

The maximum weight variation obtained was 2.50%, which falls within the acceptable weight variation range, i.e., ±5% hence passing the weight variation test. The hardness of prepared tablets was in the range of 2.94 to 3.02 kg/cm2, which falls within the limit of not < 3.0 kg/cm2

None of the tablets showed a friability value of more than 0.90% which is less than the ideal limit, i.e., 1%.

Disintegration time

The disintegration apparatus used for the study was determined by using USP (Electro lab-ED2 SAPO). It contains two basket rack assemblies. Each basket rack assembly comprises 6 glass tubes that are 3 inches long, open at the top, and held against 10 mesh screens at the bottom. Each tablet was placed in each basket tube, and the basket rack was dipped in a 1-L beaker of distilled water. The 37±2 °C temperature was maintained throughout the study (table 5).

Dispersion time

The dispersion time of polyherbal dispersible tablets was observed by placing 2 tablets in 100 ml of water in a beaker and gently stirring until dispersed completely. A smooth dispersion was obtained by passing through a sieve screen with a nominal mesh aperture (table 5).

Table No 05: Disintegration and dispersion time of polyherbal dispersible tablet Formulations Disintegration time (Min) Dispersion time (Min)

Formulations	Disintegration time (Min)	Dispersion time (Min)
PHF-1	02.18±0.51	2.50±0.65
PHF-2	02.00±0.45	3.00±0.78
PHF-3	01.10±0.10	2.00±0.45
PHF-4	01.45±0.28	2.30±0.60
PHF-5	02.08±0.62	3.18±0.82
PHF-6	01.50±0.58	3.00±0.8
PHF-7	02.06±0.70	2.55±0.71
PHF-8	02.15±0.55	3.25±0.80
PHF-9	01.55±0.60	2.24±0.58

Table 6: Stability data of the polyherbal dispersible tablet (PHF-3)

% Drug content at different storage conditions			
Time	25 °C and 60 % RH	30 °C and 65 % RH	40 °C and 75 % RH
1 Month	98.3	98.5	99.3
2 Month	99.5	99.3	98.3
3 Month	99.2	99.1	97.2

Table No 7 Dissolution study of Polyherbal formulation

TIME (MIN)	PERCENTAGE RELEASED
15	28
30	51
45	62
60	74
75	81
90	90

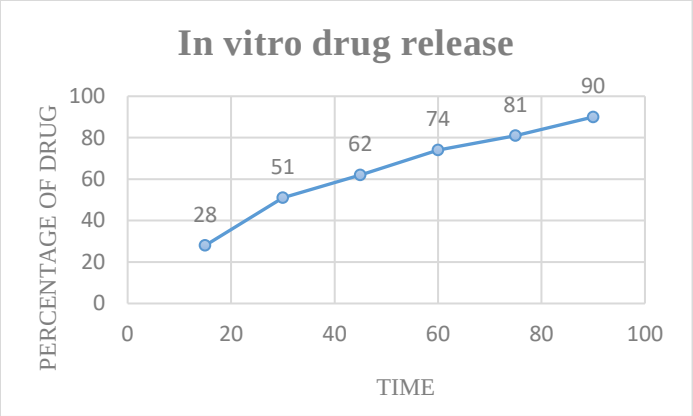
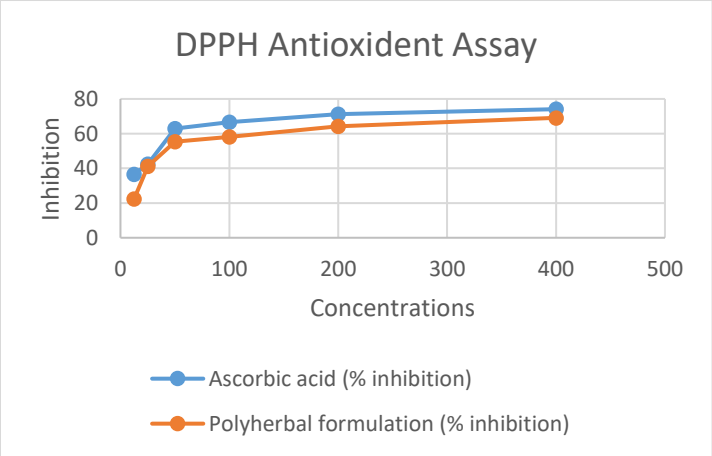


Table No. 8 DPPH activity of polyherbal formulation with reference as ascorbic acid

S. No	Concentration (µgm/ml)	Ascorbic acid (% inhibition)	Polyherbal formulation (% inhibition)
1.	12.5	36.5	22.3
2.	25	42.5	41.2
3.	50	62.9	55.3
4.	100	66.7	58.2
5.	200	71.2	64.2
6.	400	74.1	69.1
IC50		12.66	37.50



The various components of the prepared polyherbal dispersible tablet formulations are shown in table 2. The formulation containing polyherbal drugs selected for preparing dispersible tablets were identified using published standard methods viz. powder characteristics, powder micro-meristics, drug content

uniformity test, disintegration and dispersion time, and stability study for drug excipients interaction study [29]. Organoleptic properties of prepared polyherbal dispersible tablet show the yellowish-Brown in color, almost round with characteristic odors and pleasant taste (fig. 1). The average thickness of tablets 5.12±0.08 mm and average diameter 12.17±0.01 mm was recorded during the evaluation and development of tablets (Table 3) [30]. . The micrometric properties of formulations containing aqueous root extracts powder used for the preparation of polyherbal dispersible tablets observed that, the formulation PHF-4 has passed and shows all excellent properties viz. bulk density (0.35±0.03 gm/ml), tapped density (0.49±0.07 gm/ml), compressibility (28.57%), Hausner ratio (1.40±0.13), and Angle of repose (24.35±1.00 °), which was comparable as per IP standard (table 1). PHF-4 has also shown the best physical properties i.e. average weight (562.28±7.78 mg), weight variation (2.36 %), content uniformity (105.05%), hardness (2.99±0.14 kg/cm2 The disintegration time of dispersible tablets containing aqueous root extract of best polyherbal formulations PHF-4 was recorded in the acidic buffer (1.10±0.10 min) and the dispersion time of the same formulation reported as 2.00±0.45 min only rated as best formulation as compared to other formulations (table 5). The stability study of polyherbal dispersible tablets for three months at a different condition was acceptable as per IP standard (table 6), and friability (0.79%) as compared to other fast dissolving tablets containing aqueous root extract of polyherbal formulations (table 4).

CONCLUSION: The results from the angle of repose, Carr's index, and Hausner's ratio showed that the powder mixtures possess good flow properties. The physical properties of PHF-1 to PHF-9 were determined for the uniformity in weight, hardness, drug content, and friability which have complied with the official requirements, and comply with the official limits mentioned in IP 2010. The PHF-3 showed good disintegration properties and in vitro dispersion time as compared to other formulations. The FTIR spectroscopy suggests the absence of any chemical interaction between the polyherbal extract and the excipients used in the dispersible tablet. The stable peaks of the drug remained unchanged in the mixtures showing characteristic functional groups like alkynes group, aliphatic amines, alkyl halides, an aromatic group, alkanes, alcohols, and the ester possess the various medicinal properties. The PHF-3 was kept for stability studies and observed that it was reproducible even on stored for three months.

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