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Development and Standardization of a Poly Herbal Formulation

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ABSTRACT

The traditional system of medicine requires intensive investigation in quality control to compete with the modern medicine globally. The present research work on development and standardization of poly herbal capsule formulation which consist of *Ocimum sanctum, Curcuma longa, Emblica officinalis, Teriminalia bellerica, Teriminalia chebula, Piper longum, Piper nigrum,* and *Zingiber officinale*. The standardization parameters such as organoleptic evaluation, powder microscopic analysis of the poly-herbal crude drugs material and physical property analysis such as bulk density, tapped density; compressibility index and angle of repose for the formulated capsules were done. The formulated poly-herbal capsule was in the conformity to the properties evaluated and can be used as reference standard for the quality control aspect.

Keywords: Poly-herbal capsule, standardization parameter, physical property analysis, quality control.

INTRODUCTION

Herbal plants are most important for mankind for treatment and management of diseases. According to the World Health Organization (WHO), "natural plants are a plant having more bioactive molecules that have been reported their therapeutic benefits, or which are mother sources of chemo-pharmaceutical semi-synthesis." Such medicinal plants are in highly demand by the pharmaceutical companies for their bioactive ingredients [Huai et al., 2010, Husain et al., 2008, Tiwari et al., 2020].

Medicinal plants have been reported worldwide in traditional medicines for the treatment of various chronic diseases. According to various reports estimated that even modern time approximately 65-75% of the World's populations depend on medicinal plants for treatment and management of diseases [Gupta et al., 2004, Sharma et al., 1998, Bhati et al., 2014]. Herbal plants and their parts are employed in a variety of therapeutic systems for the treatment of ailments, including homoeopathy, Chinese medicine, Unani medicine, and Ayurvedic medicine.

Plant-based formulations are very cost-effective and have fewer adverse effects when used to treat ailments in herbal medicine. The guarantee of herbal product quality is a major concern for maintaining global standards. As a result, standardisation and evaluation are required. The quality and purity of the herbal product must be ensured. These standardised herbal products improve the quality of plant medicine and raise its acceptability and export quality in the global market [Shukla et al., 2019].

Poly-herbal formulation is a great idea that is suited and appropriate for the treatment and management of chronic diseases like cancer. Many studies have been reported with positive findings; however more research is needed to produce promising natural products. Therefore our goal of our current research was development and standardization of raw materials used in the poly-herbal formulation based on organoleptic, powder microscopy and physical property standardization [Jemal et al., 2006, Ferguson et al., 2004, Jo et al., 2004, Mukherjee et al., 2001].

MATERIALS AND METHODS

Plant Materials

Ocimum sanctum, Curcuma longa, Emblica officinalis, Teriminalia bellerica, Teriminalia chebula, Piper longum, Piper nigrum, and *Zingiber officinale* were among the plants collected from the local market. The plants materials are verified by Pharmacognosist, Dr. Sandeep Kumar Singh, at the Central Ayurvedic Research Institute in Jhansi, Uttar Pradesh, with accession numbers CARI/H/13212021, CARI/H/13222021, CARI/H/13232021, CARI/H/13242021, CARI/H/13252021, CARI/H/13262021, CARI/H/13272021, and CARI/H/13272021 herbal crude drugs were used to make the poly-herbal formulation. Required all of the necessary chemicals and reagents were of analytical grade purchased and used.

Standardization of poly-herbal crude drugs

Organoleptic evaluation [Garg et al., 2021, Patil et al., 2018, Shukla et al., 2019]

The organoleptic characters such as colour, odour, and taste were evaluated by spreading the powder on a clean dry sheet and investigated through the magnifying lens by repeated observation.

Powder microscopy analysis [Bisht et al., 2021, Bajaj et al., 2012, Pandeya et al., 2021]

The powder samples was treated with phloroglucinol (2% w/v) in ethanol (90%) and concentrated hydrochloric acid (1:1) and studied for their components of diagnostic value. After combining with glycerin, a sufficient amount of coarsely powdered medication was put on a glass slide. All observations were made with ocular 10x and 40x objectives, and diagnostic characteristics were photographed. Powder microscopy was used to look for lignified structures such as stone cells, calcium oxalate crystals, starch granules, epidermal cells, xylem fibres, tracheids, parenchyma cells, cuticular cell walls, essential oils, resins, lipids, and fatty oil in a poly-herbal combination.

Other parameters of standardization of poly-herbal crude drugs [Muthusamy et al., 2014, Sharma et al., 2013, Pandey et al., 2016, Shukla et al., 2019].

Other parameters of standardization of poly-herbal crude drugs are moisture content; total ash value, water soluble ash, acid insoluble ash, heavy metals, water soluble extractive, alcohol soluble extractive, and pH were among the physicochemical parameters of raw materials assessed according to WHO criteria.

pH value:

A digital pH meter was used to determine the pH of a 1 percent poly-herbal crude drugs solution by using digital pH meter.

Limit test for heavy metals:

Pharmacopoeia approaches were used for qualitative heavy metal estimation of arsenic and lead.

Extract of poly-herbal crude drugs combination [Pandeya et al., 2021]

Plant materials were purchased from local market at Indore, Madhya Pradesh, India's for the extraction of poly-herbal materials. The powdered poly-herbal plant materials was separately weighed and were taken in ratio *Ocimum sanctum* 50mg, *Curcuma longa* 50mg, *Emblica officinalis* 50mg, *Teriminalia bellerica* 50mg, *Teriminalia chebula* 50mg, *Piper longum* 50mg, *Piper nigrum* 25mg and *Zingiber officinale* 25mg and mixed well. Then after prepared poly-herbal crude drugs mixture were dipped in hydroalcohalic solvent. The extraction hydroalcohalic solvent was taken in ratio of 70:30 (ethanol: distilled water). This containers was shaked regularly basis for 15 days. The solvent was filtered and evaporated at 40 degrees Celsius in a rotary vacuum evaporator. The dried poly-herbal hydroalcoholic extract (PHAE) was obtained after filtering and evaporation. The poly-herbal extract was packed in an airtight container and kept in a cool place for further studies.

Phytochemical test

The phytochemical investigation of poly-herbal hydroalcoholic extract (PHAE) was performed, for the investigation of secondary metabolites such as alkaloids, carbohydrates, flavonoids, tannins, and steroids etc [Shukla et al., 2019].

FORMULATION

Preparation of formulation by wet granulation method [Pandeya et al., 2921, Parasuraman et al., 2014]

The poly-herbal formulation preparation began with hit and trials, with several ratios of binders and quantities of lubricants and preservatives being selected, before the technique was finally refined. Poly-herbal hydroalcoholic extract (PHAE) of *Ocimum sanctum*, *Curcuma longa*, *Emblica officinalis*, *Teriminalia bellerica*, *Teriminalia chebula*, *Piper longum*, *Piper nigrum* and *Zingiber officinale* were coarsely powdered (sieve 40), mixed in a ratio of 2:2:1, and used to make capsules using wet granulation technique with a starch (8.5% w/w) slurry. The starch (8.5% w/w) slurry was added in poly-herbal blends by geometric dilution, to obtain a homogenous damp mass. The damp mass was sieved into granules using a sieve of aperture no 80 then after damp granules were dried to

obtain a constant weight. The dried granules were then screened using aperture no 120 and 1 percent magnesium stearate was used to lubricate the granules. The granules from the optimised batch (20 percent lactose) were then put in yellow size "00" capsules in a capsule filling machine. The capsules were then removed and placed in poly bags, which were then tagged, and the samples were analysed according to the testing standards. Each poly-herbal capsule was having 500mg.

Physical property analysis of poly-herbal granules

Bulk density, tap density and Carr's index

In a 50ml measuring cylinder, a weighted quantity (15g) of poly herbal powdered ingredients was placed and the initial volume was recorded (vo). After 50 taps, the contents were volume checked and the powdered volumes were recorded (v50). This procedure was done three times, with the average determined and recorded. Fluff density (B.D.) is defined as the weight of powder divided by the volume of powder in millilitres.

T.D. = weight of powder/volume occupied by powder

Tapped density- Fluff density/ Tapped density * 100 = Carr's index (C.I.)

Carr's index values below 15 indicate excellent flowing material, whereas values between 20 and 30 indicate poor flowing material.

Angle of repose

On a burette stand, a funnel was mounted at a specific height (1.5, 2.5, 3.5 cm). On the table, a white paper was placed beneath the funnel. Slowly, the powdered substance made its way through the funnel, eventually forming a pile. The pile's radius was calculated.

The powder material's angle of repose was computed using the formula: tan = h/r = tan (h/r).

Where h is the pile's height and r is its radius.

Angles of repose of 30^{o} normally indicate a freely flowing material, while angles of 40^{o} usually indicate a poorly flowing material.

Hausner's ratio

The basic approach is to tap the powder until no more volume changes occur and measure the unsettled apparent volume, V0, and the final tap volume, Vf. The following is how the Hausner's ratio was calculated:

T.D. / B.D. is Hausner's ratio.

Between 1.00 to 1.11, Hausner's ratio indicates excellent flow, whereas greater than 1.60 indicates extremely poor flow.

Capsule Filling and Packing

A manual capsule filling machine was used to fill the capsules in a controlled environment (25°C and less than 60% relative humidity) (300 capsules in a single operation). [Mahto et al., 2022, Beg et al., 2021, Jayachandra et al., 2019] Capsules containing 500mg or more, no more than two capsules should deviate by more than 5%, and none should depart by more than 10%. The uniformity of weight test revealed that all of the medication capsules had weights that were substantially within the permissible range (Table 4). It suggests that pre-encapsulation procedures like granulation and filling the hard gelatin body were completed precisely and consistently. The filled capsules were de-dusted, sealed, and kept in bottles containing silica gel packets for moisture-free storage.

Capsule evaluation:

The poly-herbal capsules were compared to Indian pharmacopoeial standards for their description, microbiological load, and uniformity of dosage units, weight fluctuation, disintegration time, and moisture content.

Microbial load analysis:

Microbial load analysis performed to ensure that the poly-herbal capsules were safe to use, and was checked to see if the total aerobic viable count, yeasts, and moulds were within the prescribed limits, and that the microorganisms Escherichia coli, Clostridia, Salmonellae, Shigella, Pseudomonas, and Staphylococcus were not present in the final formulation.

Weight variation:

Twenty capsules were weighed separately, and the average weight was computed. Average weight-individual weight/ Average weight x 100 = Weight variance

Moisture level:

The amount of moisture in the air was measured using automatic Karl Fischer titration equipment.

Uniformity in Drug Content

An essential quality control test in the evaluation of a finished pharmaceutical product is the homogeneity of the drug content. In order to give the patient the proper amount and prevent under- and overdosing, it makes sure that a constant dose of the active ingredient is maintained between and among production batches. Under dosing will result in less than ideal results, while overdoing will have unfavorable side effects on the user.

Dissolution

The dissolution of a poly-herbal formulation preparation was investigated. In some situations, dissolution is a useful

technique for estimating absorption and bioavailability, and it can even be used to replace clinical tests in determining medication bioequivalence. Six capsules were employed in basket-style dissolution equipment using distilled water as the dissolution media. The speed was set at 50 rpm for 1 hour, and the sample was drawn every 10 minutes, with the amount of dissolved active ingredient in the solution calculated as a percentage dissolved in that time.

Stability

The stability profile of the developed poly-herbal capsule formulation was investigated under accelerated temperatures, humidity, and light intensities. Extrinsic aspects such as physical, chemical, and therapeutic changes in the poly-herbal capsule were studied, and the results were determined using the following parameters.

a) **Light:** To assess powder material deterioration, the developed poly-herbal capsule formulation was stored in various intensities of light, including sunrays, fluorescent (tube) light, UV, and infrared light.

b) **Temperature:** The effect of temperature on the stability of the created poly-herbal capsule formulation was investigated by holding all capsules at different temperatures for 30 minutes, 1, 3, and 6 hours at ambient, 35°C, 50°C, 55°C, and 65°C.

c) **Humidity:** The influence of humidity on the stability of the capsule was tested by using the full capsule at four different humidity percentages, namely 30%, 50%, 70%, and 90%.

4. RESULTS AND DISCUSSIONS

The most important aspect of the created poly-herbal formulation standardisation is quality, safety, and reproducibility. It encompasses the complete process, beginning with the harvesting of plants and other raw materials and ending with the development of a finished product using firm gelatin capsules, a standardized development of a poly-herbal mixture was developed in this study. Poly-herbal capsules were prepared using eight different crude drugs from different families, as well as different morphological plant sections and phyto-constituents.

The intensive investigation of the formulation in the alternative system of medicine is the need of the hour to fill the lacuna in the quality control of herbal drugs. The formulated poly herbal capsule is investigated for its organoleptic evaluation. The characters showed pale yellow colour with characteristic odour and bitter taste.

Standardization of poly-herbal crude drugs

Organoleptic evaluation [Garg et al., 2021, Patil et al., 2018]

Organoleptic evaluation are shown in Table 1

Powder microscopy analysis [Bisht et al., 2021, Bajaj et al., 2012, Mahto et al., 2022]

Poly-herbal combination powder microscopy study revealed the presence of starch grains; b: a prismatic crystal of calcium oxalate; c: a fragment of fibres and annular vessel; d: parenchymaous cells with anomocytic stomata; e: fibre (reticulate), f; pitted vessel attached with bunch of fibres, g; fragments of medullary rays, h; cork cells (lign (Fig. 1)

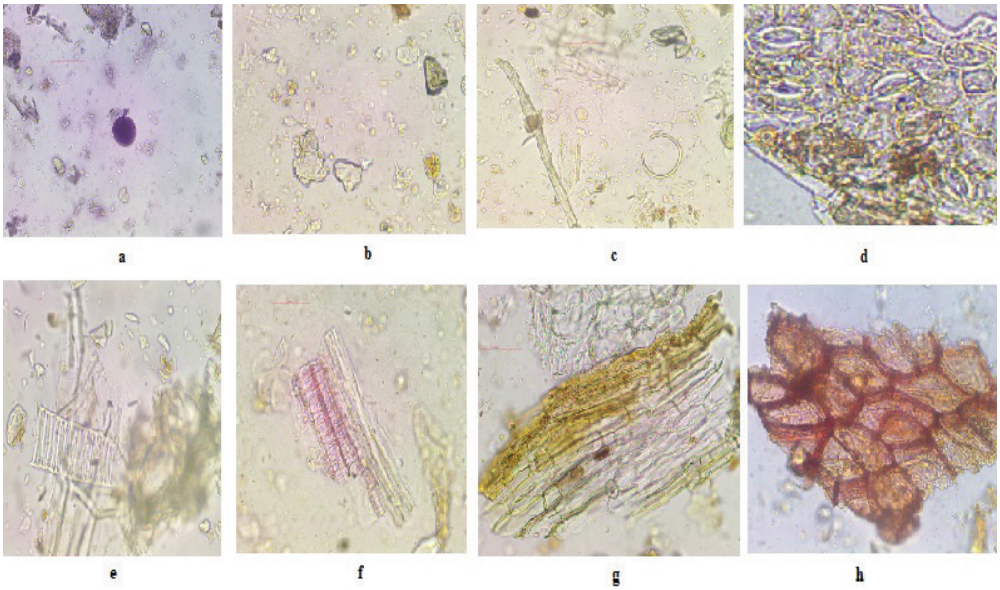

Figure 1: Powder characteristics of plants materials used in poly-herbal formulation

a: starch grains; b: prismatic crystal of calcium oxalate; c: fragment of fibres and annular vessel; d: parenchymaous cells with anomocytic stomata; e: fibre (reticulate), f; pitted vessel attached with bunch of fibres, g; fragments of medullary rays, h; cork cells (lignified).

Phytochemical investigation

Poly-herbal extract were investigated phytochemically. In the case of herbal medicine, thorough and full identification is one of the most crucial parameters because if the herbs are not properly identified, the formulation will not deliver the desired benefits. It was important to test the presence of distinct chemical compositions of the herbs before the blends were placed into the capsule dosage form. Alkaloids, Glycosides, Sterols, Triterpenes and saponins were detected in poly-herbal extract. It was indicating that this combination is suited for therapeutic activity.

The results of these phytochemical tests revealed the presence of alkaloids, tannins, and flavonoids in the extract of PHAE. The results of phytochemical investigations are shown in Table 2.

Tablet 2: Chemical composition of eight herbal plants used in the preparation of PHAE

Chemical composition	Poly-herbal formulation
Proteins	-
Lipids	-
Alkaloids	+
Glycosides	+
Sterols	+
Triterpenes	+
Tannins/Flavonoids	++
Saponins	+
Steroids	-

Standardization of prepared poly-herbal crude drugs [Muthusamy et al., 2014, Sharma et al., 2013, Pandey et al., 2016]

Moisture content, total ash value, water soluble ash, acid insoluble ash, heavy metals, water soluble extractive, alcohol soluble extractive, and acidity were among the physicochemical parameters of raw materials assessed according to WHO criteria (pH). The results of standardization of prepared poly-herbal crude drugs are shown in Table 1.

Ash value and extractive value:

Total ash, water soluble ash, acid soluble ash, and extractive values were obtained using the procedure outlined elsewhere. Loss on drying 2.55 percent, total ash 5.66 percent, acid-insoluble ash 1.28 percent, and water-soluble ash 3.51 percent, water-soluble extractive value 16.72 percent, ethanol-soluble extractive value 13.38 percent, arsenic not more than 5ppm, microbial load analysis, presence of *E.coli* (should be absent), presence of salmonella (should be absent), presence of streptococcus (should be absent), presence of pseudomonas (should be absent) and total microbial count of yeast and molds are under limit. The results are shown in Table 1.

Limit test for heavy metals:

The Limits for heavy metals were performed as per official Pharmacopeia methods. The heavy metals like as Arsenic and lead was found to be in under limit like Arsenic in 5ppm, and Lead not more than10ppm, respectively. The results are shown in Table 1.

Tablet 1: Standardization of poly-herbal capsule

Name of the test	Observations
Organoleptic characters	Brown colour powder
Colour	Brown
Odour	Characteristic
Taste	Bitter
Physiochemical parameters	7.7
pH	1.01%
Moisture content	533mg
Average weight	2.83%
Weight variation	3min25 seconds±0.21
Disintegration time (Mean ±	2.55%
SEM)	5.66%
Loss on drying	1.28%
Total ash	3.51%
Acid-insoluble ash	16.72%
Water-soluble ash	13.38%
Water-soluble extractive value	Complies
Ethanol-soluble extractive	Complies
value	113 cfu/g
Limits for heavy metals	Nil
Arsenic not more than 5ppm	Absent
Lead not more than 10ppm	Absent
Microbial load analysis	Absent
Total microbial count NMT	Absent
1000cfu/g	
Yeast and molds	
Presence of E.coli (should be	
absent)	
Presence of Salmonella (should	
be absent)	
Presence of Streptococcus	
(should be absent)	
Presence of Pseudomonas	
(should be absent)	

NMT: Not more than Result (n=3) are reported as Mean ± Standard deviation

Preparations of poly-herbal extract [Pandeya et al., 2021, Parasuraman et al., 2014, Mourya et al., 2017]

Preparation of poly-herbal extract was performed by using hydroalcohalic solvent. The yield of hydroalcohalic extract was found to be 8.1%.

EVALUATION OF POLY-HERBAL CAPSULE FORMULATION

Flow property of powdered extract [Muthusamy et al., 2014, Sharma et al., 2013, Pandey et al., 2016]

The flow quality of the powder was verified before it was filled into capsules. According to Indian Pharmacopoeia, the flow property of poly-herbal formulation was found to be the best and within acceptable limits. The results are reported in Table 3 below. The flow property of the blend to be placed in the capsule should be within acceptable limits, as determined by the following parameters. Formulation of a poly-herbal pill that was optimized

Table 3: Preformulation parameters

Parameters	Poly-herbal formulation
Bulk density	0.823g/ml
Tapped density	0.702 g/ml
Carrs index	14.7023
Hausners ratio	1.14±0.04
Angle of repose	19.04^0

Evaluation of poly-herbal capsule formulation

Granules in a "light brown" colour packed in "00" size blue capsules. The poly-herbal capsules were compared to Indian pharmacopoeial standards for their description, microbiological load, and uniformity of dosage units, weight fluctuation, and disintegration time. Brown colour, distinctive scent, and taste were discovered to have organoleptic characteristics.

pH value:

A digital pH meter was used to determine the pH and was found to be pH 7.7 of a 1 percent solution.

Microbial load analysis:

Microbiological load was found under limit as per WHO guide line limit

Weight variation:

The average weight of prepared poly-herbal formulation was found to be 533mg and weight variation 2.83 percent was found.

Moisture level:

The moisture content of prepared poly-herbal formulation was found to be 1.01 percent

Uniformity in Drug Content:

According to official method a poly-herbal capsule passes this test. The poly-herbal capsule formulations are shown the specified acceptance limit and won't result in an under dosing or overdosing effect. The consistency of the drug contents of the prepared capsules was attributed to effective granulation, particle size distribution, and homogeneous filling of the capsule shells. The test results were found to be under limit 85% to 115% range.

DISSOLUTION:

The in-vitro approach was used to investigate the optimal poly-herbal dissolving formulation up to 30 minutes; the highest cumulative drug release of a bioactive drug molecule is 98.6734 percent. The results are shown in Table 4 and Fig.2.

Tablet 4: In-Vitro Dissolution Studies

Time (min)	Abs	%CDR
0	0.0571	3.68640
5	0.3612	47.8520
10	0.4460	67.1841
15	0.5833	76.2171
20	0.6712	85.0103
25	0.7371	93.8643
30	0.7690	98.6734

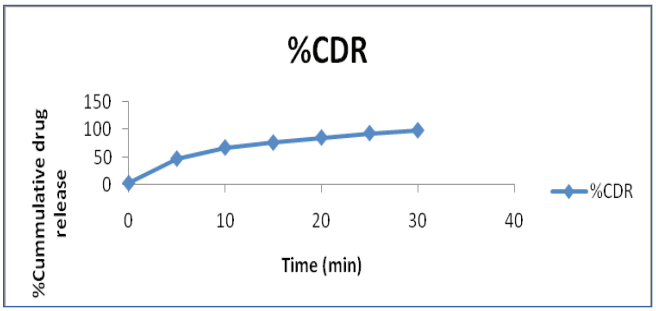

Figure 2: In-vitro %cumulative drug release of poly-herbal capsule

Stability

The stability parameters were compared after 30 minutes, 1, 3, and 6 hours of storage under accelerated temperature, light, and humidity conditions. The observation has been summarised in table 5, 6, and 7 for three Stability parameters, suggesting that there gross physical qualities do not generate any substantial change. The results are shown in Table 5, 6 and Table 7.

Tablet 5: Effect of different intensities of lights on poly-herbal capsules

Light Source	Sun light				Fluorescence				Tube light				UV Light				Infra-Red (IR)				Lamp Light			
Time of Exposure (hours)	1/2	1	3	6	1/2	1	3	6	½	1	3	6	1/2	1	3	6	1/2	1	3	6	1/2	1	3	6
600mg polyherbal capsule	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Tablet 6: Stability test of poly-herbal capsule at different temperature

Storage condition	Testing condition	Time Duration (hours)				Result
		1/2	1	3	6	
Ambient	30°C	-	-	-	-	No change during 6 hours after
Warm (30-40 °C)	35°C	-	-	-	-	No change during 6 hours after
Accelerated	50°C	-	-	-	-	No change during 6 hours after
Accelerated	55°C	-	-	-	+	Degradation start after 4 hours
Accelerated	65°C	-	-	+	+	Degradation start after 2 hours

(-) No change, (+) Degradation starts

Tablet 7: Stability of polyherbal capsule at different humidity and temperature

Temperature	30% Humidity	50% Humidity	70% Humidity	90% Humidity
30%	-	-	-	-
35%	-	-	-	-
55%	-	-	+	++
65%	-	-	++	+++

(+) Degradation (-) No Change

For the treatment of various diseases, many types of poly-herbal dosage forms have been used as medicinal agents. Traditional herbs are used to create these bioactive compounds. It may have therapeutic implications in the treatment of chronic disorders [Pandey et al., 2016, Mahto et al., 2022]. As a result, more research is needed to create the optimal herbal formulation combination. In this research work wet granulation process was used to create a poly-herbal combination capsule formulation, which was subsequently tested for quality poly-herbal product. The pre-formulation and formulation investigations of the created poly-herbal capsule solid dosage form were examined since it is highly important regardless of their medicinal content and therapeutic conditions. Angle of repose (a common characterisation method for pharmaceutical powder flow), porosity (packing geometry), Carr's index, and Hausner's ratio are some of the preformulation parameters (a measure of the inter-particulate friction). These are helpful tools for creating new poly-herbal formulations.

A value of 30° denotes 'good' flow, whereas a value of >56° denotes' very bad' flow. The flow was given an 'excellent' rating as a result of this. 14.7023 and 1.140.04 were found to represent the CI and HR, respectively. A material with a lower CI or Hausner ratio has superior flow qualities than one with a higher one. Powder flow helps to avoid the costly and time-consuming process of emptying powders that will not flow out of storage containers as well as assisting in the development of the optimal formulation and improving the product's quality and consistency.

After undergoing phytopharmaceutical review in accordance with pharmacopoeial norms, all eight herbal medications were approved as quality drugs. *In-vitro*, each poly-herbal capsule weighing 500 mg approx dissolved in 13.1415 minutes. The release of a drug from a solid dosage format in which the substance dissolved in the fluid of the gastrointestinal system is known as drug dissolution. The results showed that all six capsules disintegrated at a rate of 90 percent after 30 minutes. During an in-vitro investigation, the drug releasing pattern from the capsule shell is used to forecast the releasing sequence. The correlations between in-vitro and in-vivo results are being used to construct a tool for determining drug bioavailability and bioequivalence.

The poly-herbal capsule was determined to be almost stable after phytopharmaceutical investigations and stability testing. The poly-herbal combination of chosen plants was tested and found to be good results. It can be utilizing more accurate methodologies is needed to investigate the contents responsible for the action and the mechanism of this activity.

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Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

CONCLUSION

Thus, our findings show that the oral dosage form (500mg capsule according to the Indian Pharmacopeia) standardization parameter of poly-herbal capsule formulation was effectively evaluated by using organoleptic evaluation, powder microscopical evaluation of the raw materials used and the physical property analysis. The results obtained from the study can be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of the herbal drugs.

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