ORIGINAL RESEARCH

FORMULATION AND EVALUATION OF TABLETS FROM NAVAYASA CHURNA BY WET GRANULATION METHOD

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Submitted on: 30.06.18;	Revised on: 15.07.18;	Accepted on: 29.07.18

ABSTRACT

Navayasa churna is a well-known polyherbal Ayurvedic preparation used to treat anaemia, liver disorders, skin diseases, dyspepsia and piles. The objective of this study was to improve patient compliance, dosage uniformity and reduce adulteration of churna by formulating it into a tablet. Granulation was necessary in order to improve flow properties and organoleptic characteristics of churna. Tablets were prepared by wet granulation. Starch solution was used as binding agent, sodium benzoate as preservative, crospovidone as superdisintegrant, talc as glidant and magnesium stereate as lubricant. Pre formulation studies showed that churna is having poor flow property. Hence wet granulation technique was adopted for tablet preparation. Tablets were evaluated for hardness, friability, weight variation and disintegration time. From the results it can be concluded that patient compliance of churna can be improved by suitable formulation strategies.

KEYWORDS: Navayasa churna, Tablet, Granulation, Patient compliance

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Indian Research Journal of Pharmacy and Science; 17(2018)1522-1538;
Journal Home Page: https://www.irjps.in
DOI: 10.21276/irjps.2018.5.2.16

INTRODUCTION[1-4]

Churnas are preparations comprising of fine powders of drugs and may be simple or compound. Navayasachurna is a herbo-mineral formulation mentioned in the Ayurvedic Formulary of India with the reference of Charakasamhita (Chikitsasthanaadhyaya 16; 70-71)[1]. It has nine main constituents hence the name and lauhabhasma (iron). This churna consist of equal parts of *Cyperus rotundus*, *Plumbago zeylanica*, *Terminalia chebula, Terminalia bellerica, Phyllanthus emblica, Zingiber officinale, Piper nigrum*, *Piper longum, Embelia ribes* and the 9 parts of the Lauhabhasma (iron). Navayasa churna is used to treat anaemia, liver disorders, skin diseases, dyspepsia and piles[2].

Patients show less interest in administering churna as they stick on to throat and tongue and also because of their bitter and pungent taste[3]. In addition to that, it is difficult to carry them while travelling, dosage uniformity is poorly regulated and rate of adulteration is high[4]. In order to overcome these problems, Navayasa churna was formulated into tablets in different concentrations of superdisintegrant and binders.

Tablet was prepared by wet granulation technique. Granulation was performed to improve flow properties and organoleptic characteristics of churna. Starch solution was used as binding agent, sodium benzoate as preservative, crospovidone as super disintegrant, talc as glidant and magnesium stereate as lubricant.

MATERIALS AND METHODS[2-7]

1. Collection of Raw Materials

Raw materials (plant part for the preparation of churna) of crude drugs, *Cyperus rotundus*, *Plumbago zeylanica*, *Terminalia chebula, Terminalia bellerica, Phyllanthusemblica, Zingiberofficinale, Piper nigrum*, *Piper longum, Embeliaribes* were obtained from local market. The plant parts were identified and authenticated by Dr. Sreekala K.C, Department of Pharmacognosy, Central Institute of Medicinal Plant Research, Kottakkal.

Lauhabhasma was obtained as gift sample from Kottakkal Arya vaidyasala, Malappuram.

2. Preparation of Churna

Raw materials were washed to remove mud and other foreign matters and dried in steam tray drier. Then each ingredient was disintegrated to fine powder separately using dry disintegrator and micro pulveriser. Each powdered ingredient was weighed as per the official formula, blended and mixed and required quantity of lauhabhasma is added to produce churna as mentioned in official textbooks[2].

Determination of powder flow property of churna

a. Bulk and Tapped density

Both bulk density (BD) and tapped density (TD) was determined as per USP. A quantity of 10 gm of powder blend was introduced in to 25 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no further change in volume was noted. BD and TD were calculated using the following equations[3].

BD = Weight of the powder blend/Untapped Volume of the packing

TD = Weight of the powder blend/Tapped Volume of the packing

Table.1: Composition of Churna

Sl No.	Common Name	Botanical Name	Part used	Each 500mg of churna contains
1	Muthanga	Cyperus rotundus L.	Tuber	27.78 mg
2	Kotuveli	Plumbag ozeylanica L.	Root	27.78 mg
3	Katukkathodu	Terminalia chebula Retz.	Fruit rind	27.78 mg
4	Nellikkathodu	Phyllanthus emblica L.	Fruit rind	27.78 mg
5	Tannikkathodu	Terminalia bellerica Roxb.	Fruit rind	27.78 mg
6	Chukku	Zingiber officinale Roscoe.	Rhizome	27.78 mg
7	Kurumulaku	Piper nigrum L.	Fruit	27.78 mg
8	Thippaly	Piper longum L.	Fruit	27.78 mg
9	Vizhalari	Embelia ribes Burm.f.	Fruit	27.78 mg
10	Lauha bhasma	Iron	As it is	250.00 mg

Angle of Repose

The angle of repose of powder blend was determined by the funnel method. The accurately weighed powder blend were taken in the funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation[3].

$$\tan \theta = h/r$$

Where, h and r are the height and radius of the powder cone.

3. Preparation of Granules from Churna

To the prepared churna 10% starch solution added along with sodium benzoate (preservative) and mixed thoroughly until a coherent mass was obtained. The wet mass was then passed through sieve no.10 to obtain uniform granules. This was then dried in a steam tray drier till LOD is below 5%w/w. The dried mass is again passed through sieve no.10 to obtain granules[3].

Table.2: Composition of Navayasa tablet

Formulation code	F1	F2	F3	F4	F5
Navayasa churna (mg)	500	500	500	500	500
Sodium benzoate (mg)	0.42	0.42	0.42	0.42	0.42
Starch(mg)	48.58	42.58	39.58	33.58	35.58
Talc(mg)	18	18	18	18	18
Crospovidone (mg)	12	18	21	27	25
Magnesium stereate (mg)	21	21	21	21	21
Total (mg)	**600**	**600**	**600**	**600**	**600**

4. Preformulation Studies

a. Angle of repose

Angle of repose was determined by funnel method in which the prepared blend was poured through the sides of funnel, the lower tip of which is 2.00cm above the hard surface. The blend was poured until the upper tip of the heap touched the lower tip of the funnel. Radius of the heap (r) was measured. The study was done three times[3].

Angle of repose was calculated by the formula:

$$\Theta = \tan^{-1}(h/r)$$

where,

Θ is the angle of repose

h is the height of the pile in cm

r is the radius of the pile in cm

b. Bulk density(ρb)

About 50g of blend was weighed and filled into a graduated measuring cylinder. Initial volume was noted. Bulk density was calculated by the formula[3],

Bulk density = Weight of the granule/Initial volume of the granule

c. Tapped density (ρt)

About 50g of blend was weighed and filled into a graduated measuring cylinder and tapped 100 times. The final volume was noted. Tapped density was calculated by the formula[3],

Tapped density = Weight of the granule / Final volume of the granule

d. Carr's index

The Carr's index denotes the compressibility of a powder. It is determined by measuring bulk density and tapped density of the powder. Carr's index is calculated by the formula[3],

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

e. Hausner's ratio

Hausner's ratio is an indication of ease of powder flow. It is calculated by the formula[3],

Hausner's ratio = Tapped density / Bulk density

5. Preparation of tablets from granules

Granules were lubricated with talc and magnesium stearate by blending manually in a polyethylene bag. The lubricated granules were then compressed on a rotary tablet punching machine[3].

6. Determination of organoleptic characters of tablets

Organoleptic characters like colour, odour, taste, shape and texture of the formulations were noted[3].

7. Determination of Post compression parameters

a. Hardness test

Hardness of the tablet of each formulation was determined using Monsanto Hardness tester. It is expressed in kg/cm^2. Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated[3].

b. Friability test

The friability of the tablets was measured in a Roche friabilator (Camp-bell Electronics, Mumbai). Tablets of a sample of 20 tablets are de-dusted in a drum for a fixed time (100 revolutions) and weighed (W) again. Percentage friability was calculated from the loss in weight as given in equation as below. The weight loss should not be more than 1%. Determination was made in triplicate[3].

c. Weight variation test

20 tablets were selected randomly from the lot and weighted individually to check for weight variation[3].

d. Thickness

Thickness of the tablet of each formulation was determined using Vernier caliper. It is expressed in mm. Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated[3].

e. Disintegration test

6 tablets from each formulation were randomly selected and disintegration study was carried out in a disintegration test apparatus using 900ml of 0.1N HCl as disintegration medium. The study was continued until all parts of the tablet were passed through the sieve[3].

8. Microscopic characters

The tablet was powdered and dissolved in distilled water and observed under a microscope for the presence of diagnostic characteristic of each individual drug[3].

9. Preliminary phytochemical analysis

Preliminary phytochemical analysis was performed like test for alkaloids, flavonoids, steroids, glycosides and carbohydrates[7].

10. Physico- chemical evaluation of tablets

a. Loss on drying

About 10g of powdered tablet was weighed in a tared china dish. This was dried at 105°C for 5

hours and weighed. Drying and weighing is continued at one-hour interval until the difference between two successive weighing is not more than 0.25 per cent. Constant weight is noted when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01g difference[4].

b. Total ash

About 2g of the ground air-dried material was accurately weighed and placed in a previously ignited and tared silica crucible. The material was spread in an even layer and ignited by gradually increasing the heat to a temperature of 500-600°C until it was white, indicating the absence of carbon. The material was cooled in a desiccator and weighed. The content of total ash was calculated in mg per g of air-dried material[4].

c. Determination of acid insoluble ash

To the crucible containing total ash 25ml of dilute hydrochloric acid was added and boiled for 5 minutes. The insoluble matter was collected on an ash-less filter paper and washed with hot water until the filtrate is neutral. The insoluble matter along with filter paper was ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug[4].

d. Determination of water soluble ash

Total ash was boiled for 5 minutes with 25ml of water. The insoluble mater was collected on an ash-less filter paper, washed with water, ignited to constant weight at a temperature not exceeding 425°C. Water soluble ash was obtained by subtracting the weight of the insoluble matter from the weight of the ash. The percentage of water soluble ash was calculated with reference to the air-dried drug[4].

e. Determination of alcohol soluble extractive

5 gm of the air dried powder of the prepared tablet was macerated with 100 ml ethanol in a stopper flask and was shaken frequently during the 1st 6 hrs and allowed to stand for 18hrs. There after it was filtered rapidly taking precaution against the loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow disk dried at 105°C, and weighed and the percentage of ethanol soluble extractive was calculated[4].

f. Determination of water soluble extractive

5 gm of the air dried powdered of the prepared tablet was macerated with 100 ml chloroform water in a stopper flask and was shaken frequently during the 1st 6 hrs and allow standing for 18hrs. There after it was filtered rapidly taking precaution against the loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow disk dried at 105°C, weighed and the percentage of water soluble extractive was calculated[4].

11. Total microbial load analysis

One gram (1g) of the sample was weighed and put in a sterile test tube containing 10 millilitres of sterile distilled water to make a suspension or slurry. Serial dilution was carried out by transferring 1 ml of the suspension into the first test tube and to the next, and on up to the 5th of the five sterile test tubes containing 9 ml of sterile distilled water before inoculation.

After gradient dilution, the samples were coated on the surface of a Nutrient agar plates and incubated at 37°C for 24- 48 hr. After incubation, the colonies were counted[5].

a. Detection of *E. coli and Salmonella*

The sample was inoculated into respective enrichment broth and incubated at 37oC for 24-48

hrs. The sample was then streaked onto specific agar media and incubated at 37oC for 24-48hrs.

b. Detection of *E. Coli*

Appearance of Blue-black colonies with green metallic sheen in Eosin-methylene blue agar will give a positive result.

c. Detection of *Salmonella*

Appearance of colourless black centred colonies will appear in Salmonella-Shigella agar will give a positive result.

12. Heavy metal analysis

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid was added and boiled for 10 minutes. The mixture was cooled down and volume made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, iron, arsenic and mercury[6].

13. Test for aflatoxins

Test for aflatoxin was done as per the procedure mentioned in Ayurvedic formulary of India part 1[6].

RESULTS

Flow property of churna as well as granules were studied by determining its bulk density, tapped density, angle of repose, carr's index and hausner's ratio and found to be in passable and excellent range respectively. The results are shown in table no 3 and 4.

Organoleptic characters such as colour, odour, taste and shape of the tablet were studied and reported with descriptive terms. The results are shown in table no 5.

Post compression parameters such as hardness, friability, weight variation, thickness and disintegration study were carried out using suitable apparatus and tabulated the results in table no 6.

Microscopic characters of the best formulation (F5) for each ingredient were observed and found to be present in it. The characters of each ingredient from F5 is shown in the fig no 1 to fig no 29.

Preliminary phytochemical analysis for alkaloids, flavonoids, tannins, steroids and terpinoids of best formulation (F5) were carried out and results are shown in table no 7.

Physico – chemical analysis such as loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive values of the best formulation was carried and tabulated in table no 8.

Microbial load analysis for determining the presence of *E.coli* and *Salmonella typhi* were carried out and reported in table no 9.

Heavy metal analysis for arsenic, mercury, lead and iron in the best formulation (F5) were done and reported the results in table no 10.

Best formulation was screened for the presence of aflatoxin and reported in table no11.

Table.3: Flow properties of churna

Sl. No.	Flow property	Churna
1.	Bulk density	0.94±0.22(gm/ml)
2.	Tapped density	1.16±0.17(gm/ml)
3.	Angle of repose	36.33±0.13

Table.4: Preformulation study of granules

Formulation code	F1	F2	F3	F4	F5
Bulk density (gm/ml)	0.111	0.101	0.108	0.104	0.123
Tapped density (gm/ml)	0.147	0.142	0.135	0.139	0.135
Angle of repose	27.42	28.31	26.12	26.18	24.23
Carr's index	24.48	29.47	20.1	25.17	18.95
Hausner's ratio	1.32	1.41	1.25	1.33	1.21

Table.5: Organoleptic characters of tablets

Sl. No.	Parameter	Description
1.	Colour	Black
2.	Odour	Characteristic
3.	Taste	Slightly bitter
4.	Shape	Round

Table.6: Post compression parameters of tablet

Formulation code	Hardness (kg/cm^2)	Friability (%)	Weight variation (%)	Thickness (mm)	Disintegration time(secs)
F1	3.8 ± 0.44	0.750	600.5 ± 2.21	3.75 ± 0.32	19.40
F2	5.2 ± 0.36	0.265	600.2± 2.38	3.77 ± 0.04	19.20
F3	4.8 ± 0.27	0.495	599.8 ± 2.59	3.65 ± 0.16	20.30
F4	4.9 ± 0.22	0.247	600.7 ± 2.52	3.85 ± 0.12	21.40
F5	4.0 ± 0.35	0.740	600.2 ± 2.38	3.80 ± 0.13	17.20

Figure: Microscopic characters of tablets (F5) *Zingiber officinale*

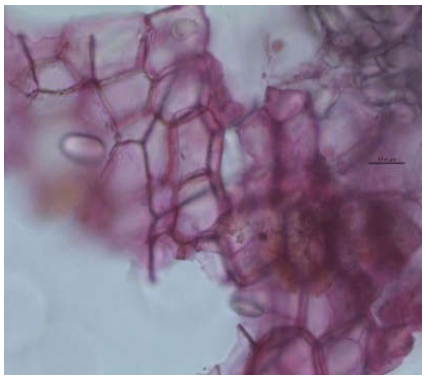

Fig. 1: Cork cells

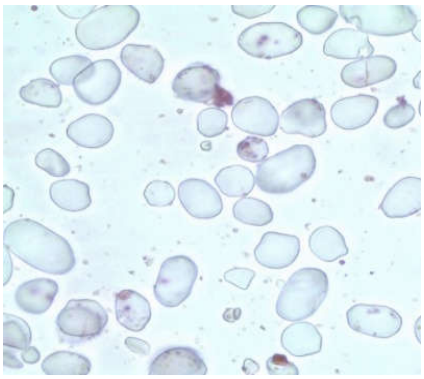

Fig. 2: Starch grains

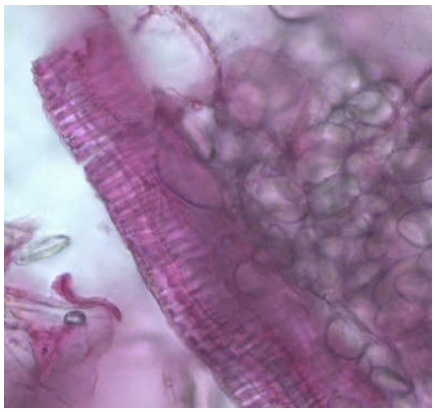

Fig. 3: Reticulate vessels

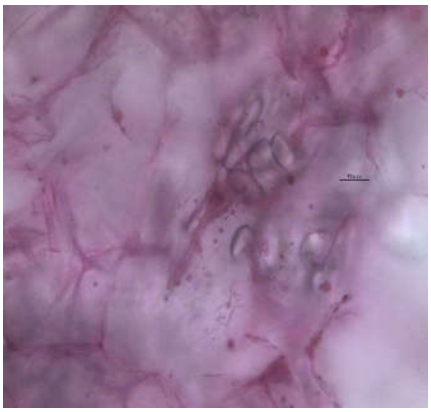

Fig. 4: Starch and oil globules

Figure: Microscopic characters of tablets (F5) *Piper nigrum*

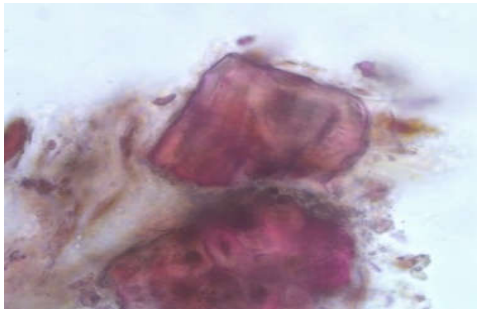

Fig. 5: Sclereids

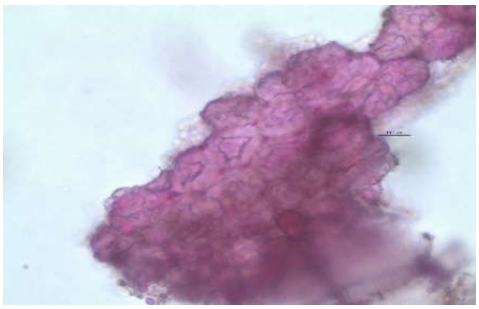

Fig. 6: Stone cells

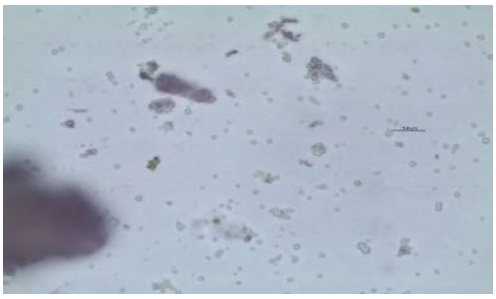

Fig. 7: Starch grains

Figure: Microscopic characters of tablets (F5) *Piper longum*

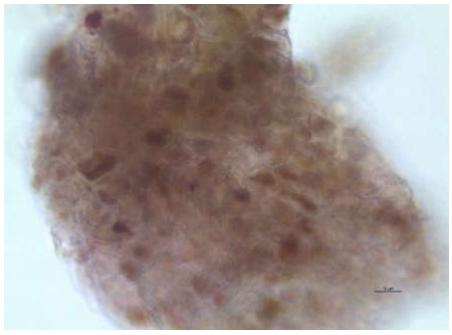

Fig. 8: Epidermis

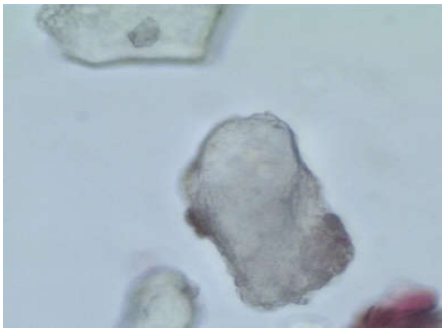

Fig. 9: Starch grains

Fig. 10: Stone cell

Figure: Microscopic characters of tablets (F5) *Cyperus rotundus*

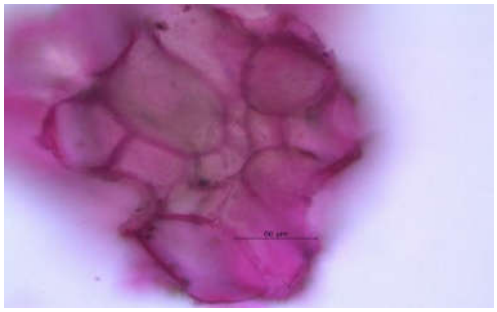

Fig. 11: Epidermis

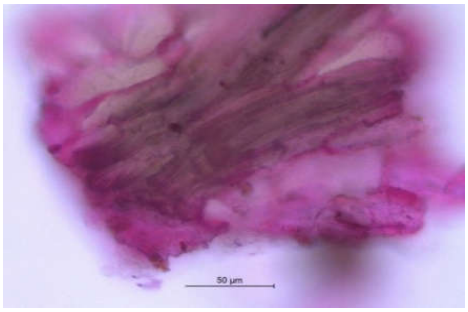

Fig. 12: Vessels

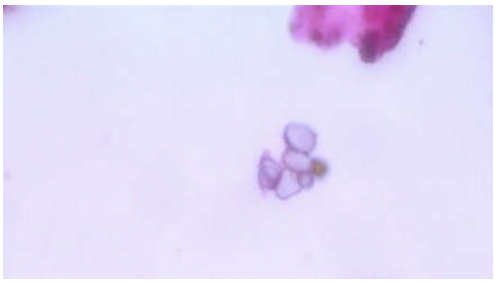

Fig. 13: Starch grains

Figure: Microscopic characters of tablets (F5) *Embelia ribes*

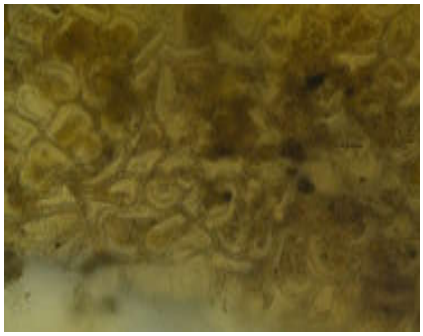

Fig. 14: Endosperm

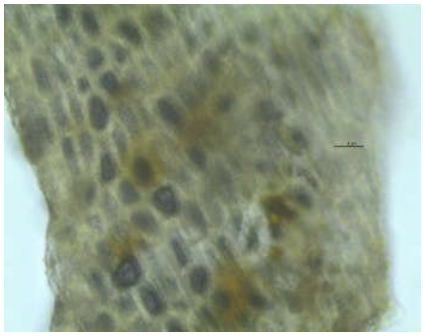

Fig. 15: Mesocarp

Fig. 16: Stone cells

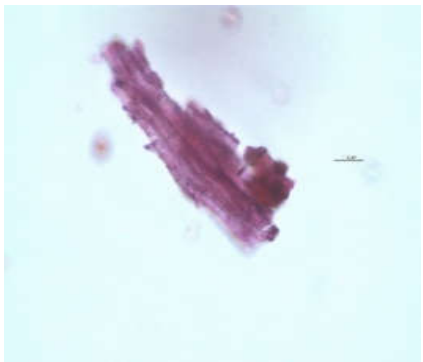

Fig. 17: Vessels

Figure: Microscopic characters of tablets (F5) *Phyllanthus emblica*

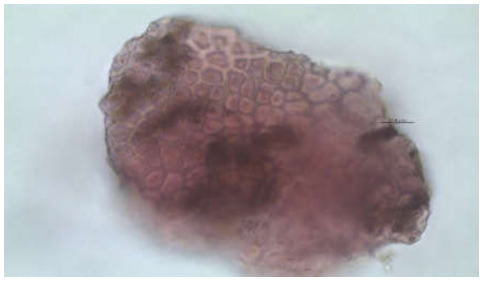

Fig. 18: Epidermis

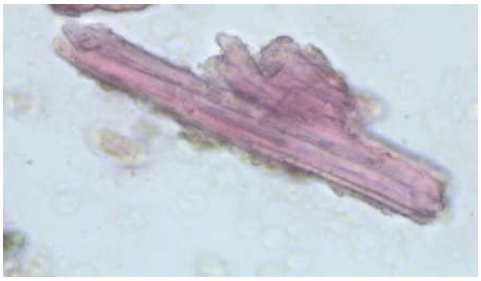

Fig. 19: Fibers

Fig. 20: Sclereid

Figure: Microscopic characters of tablets (F5) *Terminalia bellerica*

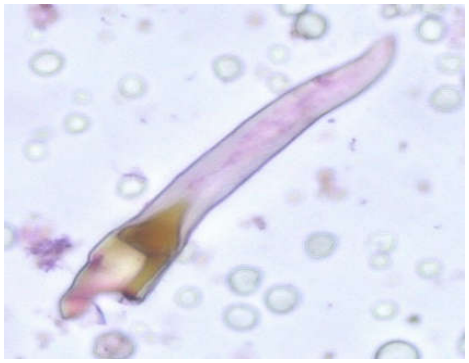

Fig. 21: Epidermal hair

Fig. 22: Reticulate parenchyma

Fig. 23: Group of stone cells

Figure: Microscopic characters of tablets (F5) *Terminalia chebula*

Fig. 24: Fibers

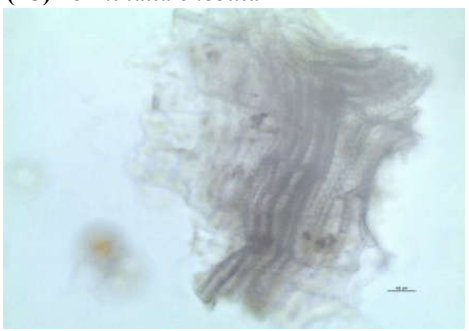

Fig. 25: Vessels

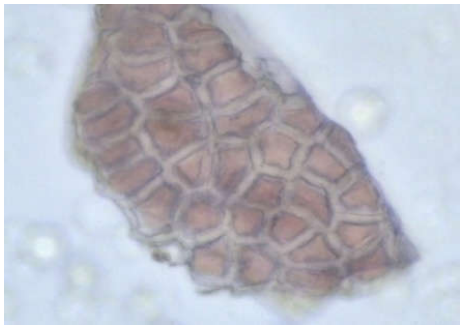

Fig. 26: Epidermis

Figure: Microscopic characters of tablets (F5) *Plumbago zeylanica*

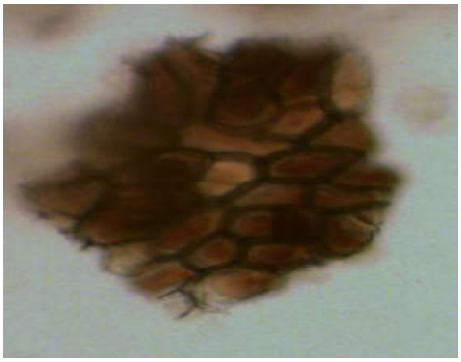

Fig. 27: Cork cell

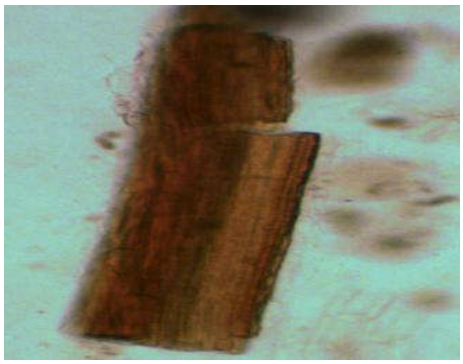

Fig. 28: Lignified fiber

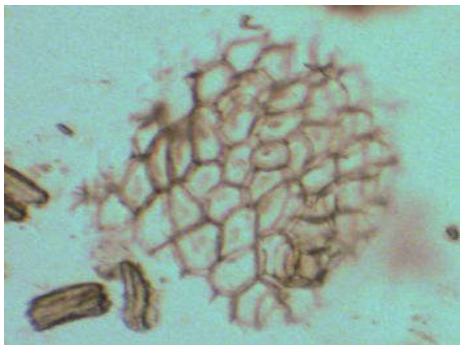

Fig. 29: Epidermal cell

Table.7: Preliminary phytochemical analysis

Sl no:	Tests	Tablet (F5)
1.	**Alkaloids**	
	Dragendroff's	+
	Mayer's	+
	Wagner's	+
2.	**Flavonoid**	
	Shinoda test	+
	Lead acetate test	+
3.	**Tannins**	
	Ferric chloride test	+
	Gelatin test	+
4.	**Steroids and triterpinoids**	
	LibermannBuchard's	-
	Salkowski's	-

Table.8: Physico – chemical evaluation

Sl.no:	Parameters	Tablet (F5) (mg)
1.	Loss on drying	1.44±0.32
2.	Total ash	50.22±0.13
3.	Acid insoluble ash	13.7±0.5
4.	Water soluble ash	4.2±0.7
5.	Alcohol soluble Extractive value	2.54±0.56
6..	Water soluble Extractive value	5.5±0.44

Table.9: Microbial load analysis of tablet (F5)

Sl.no.	Microorganism	Result	Inference as per WHO
1.	E. coli	Absent	Absent
2.	Salmonella	Absent	Absent

Table.10: Heavy metal analysis of tablet (F5)

Sl. No.	Heavy metal	Result
1.	Arsenic	<0.01 mg/kg
2.	Mercury	<0.001 mg/kg
3.	Lead	<0.01 mg/kg
4.	Iron	23.6%

Table.11: Determination of aflatoxin in tablet (F5)

Parameters	Specification	Detection Limit	Test method	Results
B1	0.5 ppm	0.004mg/kg	API Part I Vol 6	Not detected
B2	0.1 ppm	0.001 mg/kg	API Part I Vol 6	Not detected
G1	0.5 ppm	0.004 mg/kg	API Part I Vol 6	Not detected
G2	0.1 ppm	0.001 mg/kg	API Part I Vol 6	Not detected

DISCUSSION

Navayasa churna was prepared and powder flow properties of churna like bulk density, tapped density, angle of repose were done. Churna showed poor flow properties and so, to improve the flow characteristics churna was converted into granules.

For each type of formulation, blends of API and excipients were prepared and evaluated for various parameters. Bulk density was found in the range of 0.101 to 0.123 g/cm^3. Tapped density was found to be in the range of 0.135 to 0.147 g/cm^3. Using above data Carr's index was calculated and was found to be in the range of 18.95 to 29.47. From this data flow property of F5 granules are found to be good and that of F2 was found to be poor. The rest of granules were passable. The range of angle of repose was found to be between 24.23 and 28.31. Angle of repose below 25 shows excellent flow property and thus F5 granules show the best flow property. Rest all blends showed good flow properties.

Tablets were developed by wet granulation method and each tablet weighed 600mg. These tablets were then subjected to various quality control parameters like weight variation, friability, hardness and thickness. The maximum weight variation of tablet was ±2.59 % which falls in the acceptable range of 5%. Hardness of the tablet was found to be in the range of 3.8 to 5.2 kg/cm^2, F2 shows highest hardness. Friability value was not more than 0.75%. Thickness was within 4mm and thus in the acceptable limit.

Tablets were then evaluated for disintegration time. On the basis of disintegration, F5 tablets were found to be best when compared to all other formulation. The formulation F5 had disintegrated within 17.20secs.

Formulation F5 was thus found to be the best and was taken for further evaluations. F5 was subjected to microscopic evaluation. Study confirms the presence of the diagnostic characters of raw materials in Navayasa lauha churna. It also provides a suitable diagnostic tool for standardization as well as identification of adulteration. Microscopic characterization of F5 tablets revealed the presence of different types of stone cells present in amla, thippali and vizhalari, cells of perisperm (vizhalari), parenchyma with oleoresin (ginger), starch grains (ginger), epidermal cells (black pepper), epidermal cells (P. zeylanica), fibers of T. chebula.

Various microorganisms like *E. coli, Salmonella typhi* contaminate herbal drugs and cause serious health hazard for detection of microorganisms, Colonies obtained on media were subjected to suitable microbial test along with pure strains to detect their presence or absence of these organisms there by confirming the non-toxic nature of the formulation.

Navayasa tablet contain iron as an important ingredient, hence the presence and concentration of iron have to be determined. Percentage of iron present was determined by Atomic Absorption Spectroscopy and 23.6% iron was found to be present, which is not more than 33% permissible in Ayurvedic Formulary.

Heavy metals contamination is one of the major problems with plant extract or products. They are usually present in the plant formulations in extremely low concentrations, making their quantitative analysis a challenging task. The values of heavy metals such as arsenic, lead and mercury were found to be within the permissible limit.

Aflatoxin are secondary metabolites produced by *Aspergillus* species contaminate a variety of agricultural and food commodities. These mycotoxins are hepato toxins and carcinogens in human. They are classified as B1, B2, G1 and G2. According to WHO guidelines, aflatoxin should not be present in herbal formulations. The prepared tablet was evaluated for the presence of aflatoxins and no traces of aflatoxin were determined. Thus the formulation complies with the limits prescribed by Ayurvedic Pharmacopoeia Part I.

ACKNOWLEDGEMENT

The success and completion of this research work required a lot of guidance and assistance from many people. We express our obeisance to god almighty for helping us in our research work. We specially express our thanks to Kottakkal Arya vaidyasala, Malapuram for providing the ingredients required for our work. We also thank Dr. Sreekala K.S, head of Pharmacognosy Dept. Central Medicinal Plant Research for plant identification and guidance in microscopy. We extend our sincere gratitude to Care Keralam Pvt ltd, Thrissur for conducting Aflatoxin analysis. We are also thankful to Unibiosys Biotech Research Labs, Kochi for performing heavy metal analysis.

AUTHOUR'S CONTRIBUTION

AA collected the raw materials, carried out the preparation of churna and its flow property studies, preparation of granules and tablets, participated in analytical works. SPS participated in the design of the study, microbial load analysis and work coordination. RR carried out the post compression study, participated in the sequence

alignment, collected and interpreted all results and drafted the manuscript.

All authors read, criticised and approved the final manuscript.

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