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FORMULATION AND EVALUATION OF DISPERSIBLE PELLETS OF *LAGENARIA SICERARIA*

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ABSTRACT

Lagenaria siceraria (Bottle gourd) is a common name in every household. Its medicinal values were identified many years ago, and still people use this plant for many disorders. Extrusion spheronization technique was employed for preparation of the pellets, to study the effect of crosscarmellose sodium, on it. The pellets were prepared by use of combination of Avicel PH 101 and lactose that indicated good flow properties. The superdisintegrant used was crosscarmellose sodium between concentration 2 to 8%, to study the effect of it on the pellets. The superdisintegrant showed low disintegration time at low concentration, while as the concentration of it increased, it extended the disintegration time. Thus, optimum concentration needs to be designed for successful formulation. Batch D3 of 6% crosscarmellose sodium concentration showed the requisite characteristic in terms of all the evaluation parameters, with DT up to 50 to 55 seconds. Thus, use of this superdisintegrant alone, but in low concentration, can be helpful, or else combination of this with other superdisintegrants can be approached, or else new superdisintegrants can be tried. Thus, the study indicated the effect of superdisintegrant for formulation of dispersible pellets.

Keywords: Extrusion-Spheronization, Crosscarmellose sodium, *Lagenaria siceraria*, Pellets

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INTRODUCTION

The use of herbal medicines is increasing day by day. The advantages of these herbals pose greater impact to the health of human beings. There are various approaches for formulating these herbals, and one of the approaches is by converting them into dispersible form, for better patient compliance and quick onset of action. Extrusion spheronization is one of the methods for formulating dispersible pellets that encompass various advantages over the other dosage forms. *Lagenaria siceraria* [LS] is one of the herbals that are widely used, as it poses number of applications, for the health of humans. It is the fruit that has number of properties like cooling, diuretic etc. The juice of this plant is also a medicine, used for many cardiac disorders[1,2]. So, considering this fact, an attempt was made to prepare dispersible pellets for the said plant. The pellets were prepared by extrusion spheronization technique, by use of superdisintegrants.

MATERIALS AND METHODS

The plant material was purchased from local market. Avicel PH 101 was purchased from Neeta Chemicals. PVP K-25 was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, Maharashtra, India. Croscarmellose sodium (Ac-di-Sol) was obtained from Signet Chemical Corporation, Mumbai

Plant collection: *Lagenaria siceraria [LS]* that was collected from local market, was authenticated from Botanical survey of India (BSI), BSI/WRC/Tech/2014 dated 20/11/2014

PHYTOCHEMICAL ANALYSIS OF *LAGENARIA SICERARIA*[3,4,5]

Phytochemical analysis of *lagenaria* siceraria granules was carried out to test for the presence of various chemical constituents using following methods.

Test for Steroids

Salkowaski test

Chloroform (2 ml) and 2 ml of concentrated sulphuric acid were added to 2 ml of test solution, shaken and allowed to stand. Change in the colour of lower chloroform layer to red and acid layer to greenish yellow fluorescence indicates the presence of steroids.

Liebermann-Burchard reaction

2 ml test solution was mixed with chloroform (2 ml). To the solution, 2 ml of acetic anhydride and 2 drops of concentrated Sulphuric acid from the side of test tube were added. Change in colour as, first red, then blue and finally green indicates the presence of steroids.

Test for Triterpenoids

Salkowaski test

Concentrated sulphuric acid (2 ml) was added to 2 ml of test solution. The solution was shaken and allowed to stand. Change in the colour of lower layer to yellow indicates the presence of triterpenoids.

Liebermann-Burchardt Test

The 3 ml of test solution was treated with 3 ml of acetic anhydride, mixed well and then 2 ml of concentrated sulfuric acid was added from the sides of the test-tube. The development of deep red colour indicates the presence of triterpenoids.

Test for Glycosides

Balget's test

2 ml of the test solution was treated with 2 ml of sodium picrate solution. The development of yellow to orange colour indicates the presence of cardiac glycosides

Keller-Killiani test

Glacial acetic acid (3-5 drops), one drop of 5% $FeCl_3$ and conc. Sulphuric acid were added to the test tube containing 2 ml of test solution. Appearance of reddish-brown color at the junction of two layers and bluish green in the upper layer indicates the presence of glycosides.

Legals test

To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Change in color to pink or red indicates the presence of cardiac glycosides.

Borntrager's test

Dilute Sulphuric acid was added to 2 ml of test solution, boiled for a few minutes and filtered. To the filtrate 2 ml of benzene or chloroform was added and shaken well. The organic layer was separated and ammonia was added. The change in colour of ammonical layer to pink-red indicates the presence of anthraquinone glycosides.

Tests for Saponin

Foam Test

Powdered extract (10-20 mg) was shaken vigorously with water (1 ml). Development of persistent foam which is stable at least for 15 minutes indicates the presence of saponins.

Tests for Carbohydrates

Molisch's test

3 ml of Molisch's reagent was added to the 3 ml of test solution, shaken for few minutes. Then 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. The development of a purple ring at the junction of two liquids indicates the presence of carbohydrates.

Barfoeds test

Barfoed's reagent (1 ml) and test solution (1 ml) were mixed in a test tube, heated in boiling water bath for 1-2 minutes and then cooled. The appearance of red precipitate indicates the presence of monosaccharides.

Fehling's test

Fehling's A and B solutions (1 ml each) were added to the test tube and boiled for 1 minute. To this 2 ml of test solution was added and heated in boiling water bath for 5-10 minutes. Appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

Benedict's test

Benedict's reagent (1 ml) and test solution (1ml) were mixed in a test tube and heated in boiling water bath for 5-10 minutes. Change in colour to yellow, green or red indicates the presence of reducing sugar.

Tests for Alkaloids

To the dry extract (20 mg) dilute hydrochloric acid (1-2 ml) was added, shaken well and filtered. With filtrate the following tests were performed.

Mayer's test

To the 3 ml of test solution, 3 drops of Mayer's reagent (potassium mercuric iodide) was added. Appearance of reddish brown or cream precipitate indicates the presence of alkaloids.

Hager's test

To 3 ml of filtrate 4-5 drops of Hager's reagent (saturated picric acid solution) was added. Appearance of yellow precipitate indicates the presence of alkaloids

Dragendorff's test

3 ml of the test solution was mixed with Dragendorff's reagent (potassium bismuth iodide). Appearance of reddish brown precipitate indicates the presence of alkaloids.

Tests for Flavonoids

Ferric-chloride test

Test solution with few drops of ferric chloride solution shows intense green colour indicating the presence of flavonoids.

Shinoda test

To the powdered extract (10 mg), 5 ml of ethanol (95%), 3 drops of hydrochloric acid and 0.5 gm magnesium turnings were added. Change of colour of solution to pink indicates the presence of flavonoids.

Tests for Tannins

Ferric-chloride test

3 ml of test solution treated with few drops of ferric chloride solution. Development of dark colour indicates the presence of tannins.

Gelatin test

03 ml of T.S when treated with gelatin solution (3ml) gives white precipitate indicating the presence of tannins.

Tests for Proteins

Millon's test

Test solution (3 ml) and Million's reagent (5 ml) were mixed in a test tube. The appearance of white precipitate changing to brick red or dissolved and gave red color to solution on heating indicates the presence of proteins.

Xanthoproteic test

To the test tube containing test solution (3 ml), 1 ml of conc. Sulphuric acid was added. Appearance of white precipitate which turns yellow on boiling and orange on addition of NH_4OH indicates the presence of tyrosin and/or tryptophan containing proteins.

Biuret test

3 ml of the test solution was treated with 4% sodium hydroxide (3-5 drops) and 1% copper sulphate solution (3-5 drops). The appearance of blue colour indicates the presence of proteins.

Ninhydrin test

Test solution (3 ml) and 3 drops of 5% lead acetate solution were boiled on water bath for 10 min. Change in the colour of solution to purple or blue indicates the presence of amino acids

Formulation of dispersible pellets

The pellets were formulated by use of extrusion spheronization technique. The dispersible pellets were prepared by use of juice of Lagenaria siceraria, Avicel PH 101, lactose, PVP K-25, superdisintegrant-crosscarmellose sodium. For using extrusion spheronization technique, the parameters were considered to obtain an ideal formulation. All the ingredients were first sieved through 40 mesh sieve and further mixed properly. To the powder blend, the juice was added, to form wet dough mass, which was ready for extrusion. The wet mass was then extruded through extruder (VJ instrument, India) that had a 0.8mm screen, and was maintained at 50 rpm. The extrudates were further collected on butter paper and transferred to spheronizer at 1500 rpm for 10 mins (VJ instrument, India) that consisted of a cross hatched plate with each groove of length of 2 mm. The pellets were collected through spheronizer, and dried. Four formulations (D1 to D4) containing different concentrations (2-8%) of crosscarmellose sodium were used for formulation of the pellets. Each formulation contained 30g of the juice of the plant. The concentration of Avicel PH 101 and Lactose was calculated on the basis of concentration of other ingredients, and PVP K-25 was added as binder.

Evaluation of pellets[6]

This test was done by using Roche friabilator (Labindia, Mumbai, India). Weighed sample of pellets were taken with 25 steel balls, each 2 mm in diameter, and test was conducted for 100 revolutions at 25 rpm. The mass retained on 25 mesh sieve was weighed, and friability was calculated as the percentage loss of mass between the initial and final weights of sample.

Flow properties

Flow properties were evaluated in terms of angle of repose, bulk density, tapped density and Hausner's ratio.

Angle of Repose

It was determined by fixed funnel method. The pellets were poured through the funnel until the top of the conical pile just touches the tip of the funnel. The radius of the base of the conical pile was measured. The angle of repose (θ) was calculated using the following formula: $\tan \theta = h/r$ Where, θ = Angle of repose, h = Height of the cone, r = Radius of the cone base.

Bulk Density

Pellets were introduced into a dry 100 ml cylinder. The pellets were carefully leveled without compacting and the unsettled apparent volume, V_0, was noted. The bulk density was calculated using the following: formula. $\rho b = M / Vo$ Where, ρb = Apparent bulk density, M = Weight of sample, V_0 = Apparent volume of powder

Tapped Density

For this test, cylinder containing the pellets was tapped 500 times initially followed by an additional taps of 750 times until difference between succeeding measurement is less than 2% and then tapped volume, Vf was measured, to the nearest graduated unit. The tapped density was calculated, in gm per ml, using the following formula. $\rho tap = M / Vf$ Where, ρtap = Tapped density, M = Weight of sample, Vf = Tapped volume of powder.

Hausner's Ratio

It denotes the ease of powder flow. Hausner's Ratio=Tapped density (ρt) / Bulk density (ρb) Where, ρt tapped density and ρb is bulk density.

Shape Analysis

The pellets were analyzed for shape analysis by use of optical microscope. At least 20 pellets from each batch were randomly selected. The pellets were mounted on a surface of optical microscope, and the images of the pellets were captured.

Pellet Disintegration Test

The pellet disintegration in water was evaluated by a tablet disintegration test apparatus. 100 mg pellets were placed along with a plastic disc in each tube and they were inserted in the disintegration test apparatus maintained at 37°C ± 1°C. Disintegration test was carried out three times for each formulation.

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of *Lagenaria sicereria*

Plant Constituents	Test performed	*Lagenaria sicereria*
Test for Steroids	Salkowaski Reaction Liebermann-Buchard Reaction	+ +
Test for Triterpenoids	Liebermann-Buchard Reaction	+
Test for Glycosides	Balget's test Keller-Killiani test Legals test Borntrager's test	- - - -
Tests for Saponin	Foam Test	++
Tests for Carbohydrates	Molisch's test Barfoed's test Fehling's test Benedict's test	++ + + ++
Test for Alkaloids	Mayer's Reagent Hager's Reagent Dragendorff's Reagent	- - -
Tests for Flavonoids	Ferric-chloride test Shinoda test	++ ++
Test for Tannins	FeCl3 Solution Gelatin test	- -
Test for Proteins	Millon's test Xanthoproteic test Biuret test Ninhydrin test	++ + ++ +

++ Higher concentration, + Present, - Absent

For extrusion spheronization technique, Avicel PH 101 is ideal excipient, due to its property, that is required for this technique. It also possesses the requisite rheological and plastic properties needed, for it. It also possess large surface area, and porosity, that is needed for facilitation of extrusion spheronization technique[7]. Due to these properties also, the pellets formed are spherical in nature. The flow properties of the pellets indicated good flow characteristics, as shown in Table 2. The pellets were spherical as observed under optical microscope.

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Table 2: Evaluation parameters for flow property of formulations

Batches	Bulk density	Tapped density	Hausner ratio	Angle of repose (^{O})	Friability (%)
D1	0.473±0.001	0.555±0.001	1.17±0.004	26.6±0.17	0.32
D2	0.42±0.001	0.458±0.001	1.09±0.008	31.4±0.19	0.41
D3	0.431±0.0021	0.468±0.001	1.08±0.012	27.3±0.17	0.24
D4	0.486±0.001	0.565±0.001	1.16±0.008	33.6±0.5	0.36

± S.D, (n=3)

Out of the formulations, D3 can be considered as the optimized formulation, as it showed less disintegration time as compared to the other formulations. The superdisintegrant had a peculiar characteristic, that in concentration between 2 to 6%, only it showed rapid disintegration, but as the concentration of it increased further to 8% and even more, then there was increase in disintegration time[8]. This property of the superdisintegrant, may be due to the gelling property of the same[9]. The disintegration time for crosscarmellose sodium was found to be within 5 minutes, but as the concentration of it increased above 6%, the disintegration extended more than 5 mins. This difference in the observation of the disintegration time may be due to the property of fibrous nature of crosscarmellose sodium, at lower concentration and that gave the rapid disintegration of the pellets[10-11]. This property is marked at low concentration than at higher concentration. At higher concentration, the passage of fluid, wicking action and swelling takes place together, so it prolongs the disintegration time at higher concentration.

CONCLUSION

In conclusion, dispersible pellets were formulated by use of *Lagenaria siceraria.* These pellets were prepared by extrusion spheronization technique. All the pellets had good flow property and friability was less than 1%. The pellets also exhibited good disintegration time, at low concentration of crosscarmellose sodium.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest