Preliminary Characterization and Comparison of Mucilage from Leaves of \textit{Hibiscus Sabdariffa} \textit{L.} as A Tablet Binder

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Article History:
Received on: 22 Aug 2020
Revised on: 19 Sep 2020
Accepted on: 22 Sep 2020

Keywords:
Hibiscus Sabdariffa, Hibiscus Sabdariffa Mucilage, Natural Binder, Tablet Binder

Abstract
The present research dealt with the extraction and characterization of mucilage from the \textit{Hibiscus sabdariffa} leaves. Compared with normal binding agents such as starch and Poly Vinyl Pyrrolidine (PVP), the mucilage of \textit{Hibiscus sabdariffa} (HSM) was assessed for its binding properties in tablet formulations. Tablets were formulated using HSM, starch and PVP as binders at various concentration to evaluate its comparative binding efficiency. The compressed tablets were analyze for their quality control tests as per IP. The extracted HSM showed the characteristics of mucilage and good physicochemical properties. The FTIR and thermal analysis compatibility tests showed that there were no significant reactions between the drug and mucilage. Granule properties of various formulations were found to be comparable and have excellent flow characteristics. Post compression parameters suggested that tablets formulated with mucilage had better hardness and friability as that of the tablets prepared with starch and PVP. The formulations exhibited a better and more consistent release as compared to standard formulations using starch and PVP as a binder. The statistical analysis of in vitro dissolution profile by using DD solver software for difference factor (f1), similarity factor (f2), and Rescigno index ($\xi$) values also indicated promising results. The results notably indicate that binding property of HSM was at par with starch and PVP.

INTRODUCTION
The genus Hibiscus of Malvaceae family includes about 300 annual or perennial herbs, shrubs and trees species (Wang \textit{et al.}, 2012). Most of its members are commonly known in English as Roselle or Hibiscus Jamaica Sorrel. \textit{Hibiscus sabdariffa} was believed to be an Indian native plant or domesticated by western Sudan's black population, which is now widely grown in both tropical and subtropical regions (Eslaminejad and Zakaria, 2011). The plant has traditionally been used as a vegetable with a rich source of iron and minerals, well-known for its use in India, Mexico and Africa in local medicine. The leaves were used for diuretic, febrifugal, choleric and hypotensive effects, diminishing blood viscosity and stimulating peristalsis of the intestine (Morton, 1987). The extracts were recorded for the treatment of heart and nerve disease. Organic acids, Polysaccharides, anthocyanins and flavonoids are related to its context of pharmacological actions (Eggenesperger and Wilker, 1996). Research articles have reported the phytochemical and pharmacological activities of the different constituents. As polysaccharides are also one of the active ingredients found in large quantities in \textit{Hibiscus sabdariffa} ’s leaves and calyces, attention
is focused on exploring their applications other than medicinal use (Singh et al., 2016; Choudhary and Pawar, 2014). In general, polysaccharides are identified as Pharmaceutical excipients from natural sources. The mucilage content was found to be about 15 percent present in Indian strain (Rocha et al., 2014). Therefore, and the current study focused on exploring *Hibiscus sabdariffa*’s mucilage polysaccharide for its binding effect in tablet formulation where paracetamol used as a model drug.

The new leaves obtained from the plant and the HSM were extracted based on the author’s cited technique (Tavakoli et al., 2007).

**MATERIALS AND METHODS**

*Hibiscus sabdariffa* was collected from Nilgiris, Tamil Nadu. Paracetamol was obtained from Granules India Pvt. Ltd. other ingredients starch, microcrystalline cellulose, talc, methylparaben and sodium propylparaben were purchased from Loba Chem Pvt. Ltd, India, PVP from J.B. Khokhani & co, Mumbai and all other solvent used in the research were of analytical grade which was purchased from Merck.

**Collection and Authentication**

The whole plant of *Hibiscus sabdariffa* was collected and authenticated from Botanical Survey of India, Southern Regional Centre, Agricultural University Campus, Coimbatore, Tamilnadu.

**Extraction and purification of HSM**

The leaves collected were thoroughly washed with water and dried for 70 hours in Hot Air Oven at 45°C. Using mortar & pestle the dried leaves were minced and passed through sieve number #40. The powdered leaves were homogenized by soaking for 20 hours with eight times its weight of water for the release of mucilage. The solution was then boiled for half an hour at 50°C and held 1 hour in water for maximum expulsion of mucilage, using a muslin bag.
the mucilage was cuddled and filtered. The filtrate was collected, and the mucilage was precipitated with three times the amount of acetone. Further, it was washed three times with distilled water. The obtained solid colored cream was dried out under vacuum for 60 hours. The separated, dried mucilage was pulverized, then passed over sieve number #80 and prepared for subsequent testing in a desiccator.

**Hibiscus sabdariffa** characterization HSM

The purified mucilage powder has been characterized for its physical properties such as solubility, pH, loss on drying, total ash value and swelling index (Okonkwo, 2010). The methods were performed under the specification and procedure for the Indian pharmacopoeia.

Figure 6: SEM analysis of *Hibiscus sabdariffa* HSM in X 30000.

![SEM analysis of Hibiscus sabdariffa HSM in X 30000.](image)

Figure 7: FTIR spectrum of HSM.

![FTIR spectrum of HSM.](image)

The powder was further subjected to the determination of physicochemical properties such as particle size determination, bulk and tapped density, compressibility index, Hausner ratio that influences the tabletting phase (Gayathri and Sundaraganapathy, 2019). The surface morphology of mucilage powder was analyzed by emitech (K550X) sputter scanning electron microscope (SEM). The viscosity of the HSM at 1%w/v concentration was determined using Brookfield viscometer (Rupa and Banik, 2011). Thermal properties of the HSM were characterized by TGA, DTA, DSC, (SDT Q600 V20.9 Build 20). X-ray diffraction (XRD) were analyzed using a Siemens D5000 X-ray diffractometer (Siemens, Munich, Germany). DSC and XRD were also performed to identify its melting point, thermal stability and polymorphic characteristics. Paracetamol tablets were formulated with HSM, starch and PVP as the binder. Drug and excipients compatibility studies were studied.

Figure 8: FTIR spectrum of paracetamol.

![FTIR spectrum of paracetamol.](image)

Figure 9: FTIR spectrum of drug and excipients.

![FTIR spectrum of drug and excipients.](image)

Figure 10: Comparative in vitro dissolution profiles for formulation (f1 to f9).

The mixture of drug and excipients were filled in a dried plastic container and taped up. The wrapped container was stored at 37°C±0.5°C for 28 days in a stability chamber. At the end of 28 days, plastic container were removed from the stability chamber.
and identified for drug and excipients interaction studies using FTIR analysis. By Wet Granulation Method, Paracetamol granules were prepared. Drug and excipients of the various formulations f1 to f8 mentioned in Table 1 were weighed accurately with weighing balance.

Then the drug was made to pass through sieve no 40 and excipients were made to pass through sieve no 60. Then the materials were mixed for 5 minutes by placing in a transparent plastic container. Deionised water was used to prepare binder solutions with the addition of preservatives. By the slow mixing of a binding solution in a glass mortar, powder blends were granulated by kneading method.

The resultant mass was dried in a hot air oven at 30°C for three minutes. Resultant granules were made to pass through sieve no 36 and again dehydrated at 30°C for 3 minutes which was collected.
and weighed. Further, the disintegrating agent was added externally to the dried granules and blended in a plastic container to which lubricants talc and magnesium stearate were mixed well. For further processing and evaluation, granules were stored in plastic containers.

**Evaluation of granules**

The prepared granules of various formulation were evaluated for granule properties like bulk and tapped density, carr’s index, Hausner ratio and angle of repose which influences during the tablet compression process. The mentioned properties were determined using the standard procedure and formulas (Nayak et al., 2015; Priyanka and Vandana, 2013).

**Preparation of tablets**

The different group of granules were subjected to compression into tablets with an average weight of 400mg per tablet by using a rotary punch tablet compression machine.
Table 1: Composition of paracetamol tablets using HSM, starch and PVP as binding agents.

| Ingredients in mg | Formulation code |
|-------------------|------------------|
|                   | f1(4%) | f2(6%) | f3(8%) | f4(4%) | f5(6%) | f6(8%) | f7(4%) | f8(6%) | f9(8%) |
| Paracetamol        | 250    | 250    | 250    | 250    | 250    | 250    | 250    | 250    | 250    |
| Starch             | 80.8   | 76.8   | 72.8   | 80.8   | 76.8   | 72.8   | 80.8   | 76.8   | 72.8   |
| Microcrystalline   | 40     | 36     | 32     | 40     | 36     | 32     | 40     | 36     | 32     |
| Hibiscus sabdariffa mucilage (Binder) | 16 | 24 | 32 | - | - | - | - | - |
| Starch (Binder)    | -      | -      | -      | 16     | 24     | 32     | -      | -      | -      |
| Poly Vinyl Pyrrolidine (Binder) | - | - | - | - | - | - | 16 | 24 | 32 |
| Sodium methyl paraben | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Sodium propyl paraben | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Demineralised water | QS | QS | QS | QS | QS | QS | QS | QS | QS |
| Talc               | 8      | 8      | 8      | 8      | 8      | 8      | 8      | 8      | 8      |
| Magnesium Stearate | 4      | 4      | 4      | 4      | 4      | 4      | 4      | 4      | 4      |
| **Total weight**   | 400    | 400    | 400    | 400    | 400    | 400    | 400    | 400    | 400    |

Table 2: Characterization of Hibiscus sabdariffa HSM.

| Parameters                              | Observed       |
|-----------------------------------------|----------------|
| Loss on drying(%)                       | 9.26%          |
| Swelling index                          | 57.1%          |
| Bulk density                            | 0.40±0.51 g/cm³|
| Tapped density                          | 0.50±0.056 g/cm³|
| Carr’s index                            | 21.0±0.851     |
| Hausner’s ratio                         | 1.250±0.046    |
| Angle of repose(º)                      | 25.0±1.26º     |
| pH (1%w/v)                              | 6.1            |
| Total Ash(%)                            | 1.24%          |
| Water-soluble ash(%)                    | 2.7%           |
| Acid insoluble ash(%)                   | 0.15%          |
| Viscosity (1% w/v solution)             | 1.12 cps       |

Each values is the mean ± S.D. (n=3).

Evaluation of tablets

The formulated tablets were checked for the official quality control tests such as uniformity of weight, hardness, friability test, disintegration time and the assay was performed as per the standards of Indian pharmacopoeia (Shende and Marathe, 2015).

In vitro dissolution studies

In vitro drug release studies of all the formulations were carried out in USP type-II apparatus (Lab India DS 8000) using phosphate buffer pH 5.8 as dissolution medium maintained at 37±2ºC temperature rotated at 50rpm for 30minutes. About 2 ml sample was withdrawn at 5, 10, 15, 20, 25, and 30 minutes time intervals from the basket and the setup, was kept under sink condition (Ngwuluka et al., 2010). The obtained sample was filtered by Whatman filter paper and diluted with 100 ml of phosphate buffer pH 5.8 and analyzed for the percentage drug release using UV Visible spectrophotometer (UV Probe Software) at 243 nm (Pant et al., 2015).
Table 3: Evaluation of the formulated granules.

| Binders & Formulation Code Parameters | HSM | STARCH | PVP |
|---------------------------------------|-----|--------|-----|
| Uniformity of weight (%)              | 400.1±0.12 | 401.4±0.1 | 400.1±0.1 |
| Hardness (kg/cm²)                     | 4.5±0.38 | 5.5±0.32 | 5.0±0.41 |
| Thickness (mm)                        | 2.3±0.01 | 2.1±0.01 | 2.1±0.01 |
| Diameter (mm)                         | 10.14±0.04 | 10.12±0.01 | 10.14±0.01 |
| Friability (%w/w)                     | 0.97±0.01 | 0.98±0.01 | 0.98±0.01 |
| Disintegration time (min)             | 2.5±0.32 | 6.2±0.35 | 13.28±0.35 |
| Assay (%)                             | 99.7±0.32 | 99.6±0.52 | 98.9±0.35 |

Table 4: Evaluation of tablets using different binding agents.

| Binders & Formulation codes Parameters | HSM | STARCH | PVP |
|---------------------------------------|-----|--------|-----|
| Uniformity of weight (%)              | 400.0±0.12 | 401.4±0.1 | 400.1±0.1 |
| Hardness (kg/cm²)                     | 4.5±0.38 | 5.5±0.32 | 5.0±0.41 |
| Thickness (mm)                        | 2.3±0.01 | 2.1±0.01 | 2.1±0.01 |
| Diameter (mm)                         | 10.14±0.04 | 10.12±0.01 | 10.14±0.01 |
| Friability (%w/w)                     | 0.97±0.01 | 0.98±0.01 | 0.98±0.01 |
| Disintegration time (min)             | 2.5±0.32 | 6.2±0.35 | 13.28±0.35 |
| Assay (%)                             | 99.7±0.32 | 99.6±0.52 | 98.9±0.35 |

RESULTS AND DISCUSSION

Extraction and characterization of HSM

The yield was approximately about 21 g HSM was obtained from 200 g of leaf powder which was found to be 10%. The results of the characterization of HSM were observed and were presented in Table 2. The result indicated that the creamy white amorphous mucilaginous powder of HSM was sparingly soluble in water; solubility was found to increase with the rise and observed to form viscous dispersion and almost insoluble in other organic solvents. And viscosity was 1.12 cps. Since the pH value of this HSM is near to neutral, it may be less irritating on GIT which suits for uncoated tablets. Loss on drying and swelling index was found to be 9.26% and 57.1% respectively.

Total ash value was indicated as 1.24% in which water-soluble ash and insoluble acid ash was 2.7% and 0.15% respectively. Carr’s index determined the flow properties of HSM powder; Hausner’s ratio and angle of repose were found to be >21.0, >1.25, and 25°-30° respectively, which indicates good flow properties.

Thermal analysis

Thermogravimetric analysis (TGA)

The thermogravimetric curve of HSM was presented.
Table 5: *In vitro* drug release of tablets using HSM, starch and PVP as binders.

| Binders & Formulations code (Dissolution time (mins)) | HSM | STARCH | PVP |
|------------------------------------------------------|-----|--------|-----|
|                                                      | f1(4%) | f2(6%) | f3(8%) | f4(4%) | f5(6%) | f6(8%) | f7(4%) | f8(6%) | f9(8%) |
| 5                                                    | 29.8 ± 0.17 | 21.6 ± 0.13 | 19.8 ± 0.11 | 30.6 ± 0.13 | 25.2 ± 0.15 | 21.6 ± 0.11 | 32.4 ± 0.16 | 25.2 ± 0.14 | 19.8 ± 0.12 |
| 10                                                   | 42.4 ± 0.12 | 34.1 ± 0.17 | 30.4 ± 0.11 | 43.2 ± 0.12 | 36.0 ± 0.15 | 32.4 ± 0.13 | 45.0 ± 0.17 | 30.6 ± 0.15 | 27.0 ± 0.12 |
| 15                                                   | 56.9 ± 0.17 | 45.2 ± 0.13 | 41.6 ± 0.12 | 54.1 ± 0.18 | 43.3 ± 0.17 | 45.0 ± 0.12 | 52.3 ± 0.17 | 34.3 ± 0.17 | 39.6 ± 0.12 |
| 20                                                   | 71.3 ± 0.15 | 56.7 ± 0.16 | 50.1 ± 0.11 | 68.5 ± 0.17 | 59.5 ± 0.14 | 52.3 ± 0.12 | 70.3 ± 0.17 | 50.5 ± 0.17 | 46.9 ± 0.12 |
| 25                                                   | 83.4 ± 0.17 | 66.3 ± 0.18 | 59.8 ± 0.13 | 79.4 ± 0.16 | 72.1 ± 0.17 | 64.9 ± 0.10 | 77.6 ± 0.17 | 63.1 ± 0.17 | 54.1 ± 0.12 |
| 30                                                   | 92.1 ± 0.18 | 72.6 ± 0.15 | 65.1 ± 0.12 | 91.9 ± 0.15 | 84.8 ± 0.12 | 75.8 ± 0.10 | 90.3 ± 0.14 | 70.4 ± 0.13 | 65.0 ± 0.12 |

Cumulative % drug release

in Figure 1. It clearly shows, weight loss corresponding to the loss of water around 25 — 190°C. The HSM underwent 9.26% weight loss at 65.21°C. It implies that HSM has excellent thermal stability. The curve also shows that the HSM did not decompose before 200°C. The decompose starts at 207.34°C. So the water is formed by intra, and inter molecular condensation of HSM hydroxyls are the main products of decomposition at a temperature below 450°C.

**Differential scanning colourimetry (DSC)**

The HSM undergoes glass transition temperature at 190.84°C (1.132 J/G). The continuous (broad) endothermic transition that precedes the glass transition is symptomatic of moisture loss in the sample. The decompose starts at 313.03°C (4.973 J/G).

**Differential thermal analysis (DTA)**

The DTA curve indicates that HSM undergoes crystallization at 56.71°C (0.1904%/°C) temperature. The HSM started to melt at 229.59°C (0.2773%/°C) based on the analysis conducted using DTA. The DSC and DTA curve were represented in Figure 1. The spectra obtained by XRD, the HSM show that the existence of frequent halos with weak peaks which signify the amorphous nature of the material. The result was represented in Figure 2.

**Scanning Electron Microscopy**

The surface morphology of HSM was observed under the scanning electron microscope (SEM). Figures 3, 4, 5 and 6 represents the SEM photographs of HSM at different magnifications. The images of *Hibiscus sabdariffa* HSM revealed that the surface of particles is habitually seen as aggregates of rough, irregular size and shape which were fibrous, which indicates the amorphous nature of the material.

**Formulation of Paracetamol tablets with HSM, starch and PVP as a binder**

**Drug and excipients compatibility studies**

The FTIR spectrum of the HSM, drug and the physical mixture were shown in Figures 7, 8 and 9 indicates no change in the characteristic peaks of the drug. Hence, it reveals that there was no interaction between the drug and excipients.
Evaluation of formulated granules

The results of the flow properties of prepared granules of different batches were presented in Table 3, and the values indicated that the flowability ranges were found to be getting reduced with the increase in the concentration of the HSM. Slight variation in the flow was observed with the granules prepared using starch and PVP. All the granules exhibited excellent flow as the values of Carr’s index, Hausner’s ratio and angle of repose of all the granules were found to be <23, <1.25 and 25°-30° respectively.

Evaluation of tablets using HSM, starch and PVP as binding agents

All the nine batches of tablets prepared using HSM, starch and PVP as binding agents were evaluated for the quality control test for tablets and the results were compared. The values were presented in Table 4. All the batches of tablets exhibited an excellent uniformity in content. The hardness of the tablets increased with increase in the percentage of binding agent. The formulation with 8% HSM was hard compared with 4% and 6%. The percentage of friability also decreased with an increase in binder concentration. The disintegration time of tablets for all the formulations was found to be within limits. It was also observed that the disintegration time increases with an increase in the concentration of the binder (4% to 8%). This behavior can be attributed to the swelling properties of the HSM.

In vitro dissolution studies of various formulations

In vitro dissolution profile of tablets was shown in Table 5 and Figure 10. This study showed that the drug release from the tablets prepared using the HSM with 4% and 6% concentrations were found to be more than 90% and 70% in 30 minutes. The percentage of drug release was found to increase with a decrease in the concentration of HSM. Similar data were observed for the formulations f4 to f9 containing starch and PVP as a binder. The percentage drug release of f1, f4 and f7 were more compared with f2, f5 and f8, also the release was found to be getting reduced for formulations f3, f6 and f9. The results of in vitro drug release indicate that the concentration of the binder influence the drug release. The increase in the ratio of the binder leads to a decrease in drug release from the tablets.

Statistical analysis

Statistical examination of in vitro dissolution profile comparison of HSM with starch and PVP as binding agents were performed using DD solver software for difference factor (f1), similarity factor (f2), and Rescigno index (ξ) values. The results were presented in Table 6, and the graphs were indicated in Figures 11, 12, 13, 14, 15 and 16. The difference factor (f1) were below 15, the similarity factor (f2) was above 50 and Rescigno index was almost 0.

CONCLUSIONS

It can be therefore concluded that the Hibiscus sabdariffa mucilage behaves as a non-starch polysaccharide which has the characteristic as a binder. It can show a significant binding efficiency compared with starch and PVP and natural and synthetic binder for tablet formulations. Further, this work can be extended with in vitro toxicity study for the confirmation of the safety and also sustaining the action of this HSM at higher concentration can be predicted.

ACKNOWLEDGEMENT

We would like to deliver our sincere thanks to the Chairman, Karpagam institutions for providing us with the facilities to carry out the research work on these premises.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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