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Isolation of Natural Binding Agent from Barley Seeds and Its Characterization

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Abstract

Development of new excipients is time consuming involves tedious procedures and highly expensive. Instead, identification of new uses for the existing substances is relatively and less time consuming. The intention of present study was designed for isolation and characterization of powder from the Hordeumvulgare seeds and explores its use as pharmaceutical binding agent. The barley powder was investigated for purity by carrying out chemical tests for different Phytochemicals constituents and Starch,Flavonoids, Phenol were found to be present. The powder was further characterized for physical and flow properties. Powder has good swelling index 12.7%, PH 6.9, Melting point 134, Moisture absorption 8%, Loss on drying 7.5%. The powder had good flow property as Carr's Index 15.27, Angle of repose 29.35 and Hausnor's ratio 1.15. From the study, it indicates that Hordeumvulgare seeds powder has satisfactory pH and physicochemical Properties, which can be used as pharmaceutical Binding agent in formulating various dosage form.

***Keywords*— Barley; Binding agent; Flow Properties; Starch.**

I. INTRODUCTION

Barley (*Hordeumvulgare* L.) is the 4th most important cereal after maize, rice, and wheat in terms of production quantity and cultivation areas. The world area harvested for barley production has kept steadily decreasing since the 1980s, though the production quantity has increased during the last 5 y. The annual production reached over 144 million tons in 2014, and the top producers in terms of production quantity are Russia, France, Germany, Australia, and Ukraine (FAOSTAT **2017**). Barley has a great adaptability to harsh environmental conditions and can thrive on marginal lands. It is cultivated across a wide range of production areas in the world such as at the high altitudes of the Himalayas and near the Arctic Circle (Horsley and Hochhalter **2004**). There is a great diversity in the morphological forms of barley, which include spring, winter, 6-row, 2-row, awned, awnless, hooded, covered and naked (hull-less), feed (forage and grain), malting, and food type (Stanca and others **2016**). Novel genetic mutants have been continuously characterized (Shaik and others **2016**).

Barley has been cultivated by humans for over 10000 y (Stanca and others **2016**). In modern days, barley is mainly used as animal feed and in malting for whisky and beer production (Stanca and others **2016**). There is growing interest in using barley for food production due to its various health effects, such as lowering blood cholesterol, regulating glycemic index, and antioxidant activity (Baik and Ullrich **2008**). Functional components of barley responsible for these health benefits include β-glucans, tocols (such as tocotrienols and tocopherols), polyphenols (such as phenolic acids, proanthocyanidins, and catechins), and others (Baik and Ullrich **2008**). Barley has been used to formulate various "healthy" food products such as pasta and bread (Stanca and others **2016**). The major component of barley kernel is starch that may amount to over 70% of the dry weight (Asare and others **2011**). Therefore, it may be expected that the quality of barley-based food/feed products can be much affected by the starch properties. Starch is also a by-product of the barley fractionation process for β-glucan production (GAO and others **2009**). Understanding the starch properties provide a basis for value-added processed products containing barley.

Recently produced novel starches from genetic mutants such as "amylose-only" genotype provide various possible applications for the food and other industries (Sagnelli and others **2016**). A systematic review on various aspects of the starch is needed to support the current exploitation of barley as a sustainable crop.

A previous review on barley starch has systematically summarized the literature published up to the year 2004 (Vasanthan and Hoover **2009**). There has been much development in our understanding of various aspects of this starch during the last decade or so. More genetic resources have been assessed and novel genotypes have been developed. New experimental tools have been used for starch analysis and conceptual breakthroughs have been attempted. Various types of starch modifications have been made to create a wide range of functionalities. This review updates the isolation methods, chemical composition, granular and chemical structures, physicochemical properties, and chemical/physical/enzymatic modifications of barley starch. The basics of starch have been well reviewed previously and are, therefore, not covered here (Vasanthan and Hoover **2009**; Pérez and Bertoft **2010**). A number of recent reports cover the impact of genetic mutation and biosynthetic enzymes on starch properties. The roles of diverse starch biosynthetic enzymes have been well reviewed previously and are not covered in this review (Nakamura **2015**; Tetlow and others **2015**). Readers, especially those who are new to the starch research field, are strongly encouraged to refer to these previous publications to gain the necessary background knowledge. The scope of barley starch topics reviewed here is very wide, and numerous concepts and many nomenclatures (especially related to the amylopectin molecular and internal structure) of starch are employed. This may present a challenge to the readers even if they have researched on starch.

1.1 Biological source:

Barley consist of Hordeumvulgare (Species: H. vulgare Genus: HordeumFamily:PoaceaeKingdom:Plantae)

1.2. Geographical distribution:

Wheat is widely cultivated cereal, spread from 57ºN to 47ºS latitude. Hence, wheat is cultivated and harvested throughout the year in one country or other. China, India, Russian federation, USA, France, Canada, Germany, Pakistan, Australia and Turkey are most important wheat growing countries. In India, UP, Punjab, Haryana, MP, Rajasthan, Bihar, Gujarat, Maharashtra, Uttaranchal and West Bengal are the important wheat cultivating states.

1.3. Organoleptic Characters:

Colour: grey or brown.

Odour: none

Taste: Mucilaginous, bland.

Size: 65mm to 1.2 cm in length and 1-2mm in width.

1.4. Uses:

- High cholesterol. Research shows that taking barley reduces total cholesterol and "bad" low-density lipoprotein (LDL) cholesterol. The benefit might depend on the amount of barley taken.
- Stomach cancer. Some research suggests that eating dietary fiber, including barley, reduces the risk of getting stomach cancer.
- Colon cancer (colorectal cancer). Eating dietary cereal fiber, including barley fiber, does not seem to reduce the risk of developing colon cancer.
- Inflammatory bowel disease (ulcerative colitis). Early research shows that eating food containing germinated barley daily for 4-24 weeks reduces the symptoms of ulcerative colitis. Other research shows that taking a specific product (Profermin) containing barley and other ingredients for 8 weeks reduces symptoms of ulcerative colitis and increases the chance of disease remission.
- **Weight loss**. Early research in Japanese women shows that eating white rice mixed with barley high in beta-glucans can decrease hunger and the amount of food eaten at the next meal.

1.5. Side Effects & Safety

Barley is LIKELY SAFE for most people when taken by mouth appropriately. In some people, barley can cause stomach upset. In some adults and children, barley can cause an allergic reaction after eaten, inhaled, or applied to the skin. Symptoms may include skin rash and difficulty breathing.

- Pregnancy and breast-feeding: Barley is LIKELY SAFE when taken by mouth during pregnancy in amounts commonly found in foods. However, barley sprouts are POSSIBLY UNSAFE and should not be eaten in high amounts during pregnancy.There is not enough reliable information about the safety of taking barley if you are breast-feeding. Stay on the safe side and avoid use.
- Celiac disease or gluten sensitivity: The gluten in barley can make celiac disease worse. Avoid using barley.
- Allergies to cereal grains: Consuming barley might cause an allergic reaction in people who are sensitive to other cereal grains, including rye, wheat,

oat, corn and rice. An allergic reaction is also possible in people allergic to grass.

- Diabetes: Barley might lower blood sugar levels. Your diabetes medications might need to be adjusted by your healthcare provider.
- Surgery: Barley might lower blood sugar levels. There is a concern that it might interfere with blood sugar control during and after surgery. Stop using barley at least 2 weeks before a scheduled surgery.

II. MATERIALS AND METHODS

1.1. ISOLATION OF BARLEY

The barley seeds were soaked in water for 7 days and the exudate polysaccharides were expressed and precipitated in acetone. The obtained powder was dried at 40◦ in tray dryer. The resultant mass was powdered and calculated for its yield .The yield was found to be 22±2.452%. The powder was stored in air tight container for carrying further studies.

1.2. Characterization of selected barley:

1.2.1. For Barley Powder:

Barley powder was characterized by various tests of identification.

2.2.2. Determination of purity of gum:

To determine the purity of gum tests for alkaloids, carbohydrates, flavonoids, steroids, saponins, tannins and phenols were carried out.

2.2.3. Organoleptic Evaluation:

The Organoleptic evaluation refers to the evaluation of color, odor, shape, taste and special features which include touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

2.2.4. Swelling index:

Swelling index of powder polysaccharide was determined by using modified method reported. One gram of powder (#100 mesh passed) was accurately weighed and transferred to a 100 ml stoppered measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 ml mark with distilled water. The cylinder was stoppered, shaken gently and set aside for 24 hours. The volume occupied by the gum sediment was noted after 24 hours. Swelling index (SI) is expressed as a percentage and calculated according to the following equation.

SI= [(Final volume – Initial volume)/Initial volume] *100

2.2.5. Solubility:

Solubility of barley powder was checked with different solvents such as water, hot water, acetone, ethanol, methanol, ether, chloroform.

2.2.6. Melting point:

The powdered sample of barley was transferred into a capillary tube and by using melting point apparatus melting point was determined.

2.2.7. Moisture Absorption:

The powder was weighed accurately and placed in a desiccator. After 3 days, the powder was taken out and weighed. The percentage of moisture uptake was calculated as the difference between final weight and initial weight with respect to initial weight.

2.2.8. Loss on Drying (LOD):

Moisture content of barley powder was determined by loss on drying method. Accurately weighed 20 g sample was heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture on drying was calculated using formula given below.

LOD (%) = (Weight of moisture in sample / Weight of sample before drying) × 100

2.2.9. pH of Powder:

The pH of 1% w/v dispersion of the powder was determined using a digital pH meter.

2.2.10. Thermal Stability:

A sufficient quantity of the banana powder was taken in a petri dish and kept at successive higher temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, 110°C, 120°C, 130°C and 140°C). The temperature at which the banana powder showed a change in colour was noted.

2.3. Flow Properties of selected Polymers:

a. Determination of particle shape distribution:

Barley powder was dispersed in glycerin and a smear of the dispersion was made and examined under microscope. The shapes of particles were measured using a calibrated eyepiece micrometer.

b. Bulk density:

Density is defined as mass per unit volume. Bulk density, ρ_b is defined as the mass of the powder divided by the bulk volume and is expressed as g/cm^3. It depends upon particle size distribution, particle shape and the particles adhere together. Apparent bulk density (ρ_b) was determined by pouring the blend into a graduated cylinder. The bulk density was calculated using the formula.

$$\rho_b = \frac{M}{V_b}$$

Where, $\rho_b$is bulk density, V_b is bulk volume, M is the weight of the powder.

c. Tapped density:

The measuring cylinder containing a known mass of blend was tapped for a 100 times using density apparatus. The minimum volume (V_t) occupied in the cylinder and the weight (M) of the blend was measured. The tapped density (ρ_t) was calculated using the formula.[61]

$$\rho_t = \frac{M}{V_t}$$

Where, $\rho_t$is tapped density, M is weight of the powder, $V_t$is tapped volume.

d. Angle of repose:

Angle of Repose was determined using funnel method. The blend was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and angle of repose (θ) was calculated using the form[62]

$$\tan\theta = \frac{h}{r}; \quad \textbf{therefore}; \ \theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where, θ is Angle of Repose, his height of Cone, r is Radius of cone

Table 1: Angle of repose

Angle of repose (°)	Type of flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

e. Compressibility Index:

The simplest way for measurement of free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility index (*I*) which is calculated as follows (equation-1).

$$I = \frac{\rho_t - \rho_b}{\rho_t} * 100 \ldots\ldots\ldots\ldots\ldots\ldots (eq.\ 1)$$

Where it is compressibility index, $\rho_t$is Tapped Density, ρ_b is Bulk Density.

Table 2. Compressibility index

Carr'sindex (%)	Type of flow
<12	Excellent
12.0-16	Good
18-21	Fair to passable
23-35	Poor
33-38	Very poor
>40	Extremely poor

f. Hausner's Ratio:

Hausner ratio (HR) is an indirect index of ease of powder flow. It is calculated by the following formula.

$$HR = \frac{\rho_t}{\rho_b}$$

Where HR is Hausner's ratio, ρt is tapped density, ρb is bulk density.

Lower Hausner's ratio (< 1.25) indicates better flow properties than higher ones (> 1.25).

III. RESULT

3.1. PHYSICOCHEMICAL CHARACTERIZATION OF BARLEY POWDER

3.1.1. Organoleptic Evaluation

The polysaccharide was characterized by various organoleptic properties such as colour, odour, taste, shape, touch and texture and shown in **Table 3**.

Table 3: Organoleptic evaluation of the powder

S. No.	Property	Result
1.	Color	Light brown
2.	Odour	Odour less
3.	Taste	Tasteless
4.	Shape	Irregular
5.	Touch and Texture	Hard and Rough

3.1.2. Phytochemical screening of the powder

The basic Phytochemical screening tests for carbohydrates, alkaloids, steroids, flavonoids saponins, tannins and phenols were carried out and shown in **Table4**. The tests indicated the absence of alkaloids, steroids, flavonoids, saponins, tannins and phenols. Only carbohydrates were found to be present.

Table 4: Determination of purity of powder

S. No.	Tests for Powder	Result
1.	Test for alkaloids Dragendrof's Test, Mayer's test	Present
2.	Test for Glycosides: Keller-Killiani test, Legal Test	Absent
3.	Test for Carbohydrates Molisch test, Barfoed's test, Benedicts test	Present
4.	Test for Flavonoids Shinoda test, Zinc/HCl reduction test	Present
5.	Test for Tannins/ Phenols Ferric chloride test, Gelatin test	Present
6.	Test for Starch Iodine Test	Present

3.1.3. Solubility Profile of powder

The solubility profile of the powder was found to as shown in **Table 5**. In strong Dimethyl sulfoxide after 5 minutes sonication at room temperature it was soluble. The polysaccharide was insoluble in acetone, alcohol, ether, chloroform. It was found to form a gel in Hot water.

Table 5: Solubility behavior of the selected powder

S. No.	Solvents	Result
1.	Dimethyl sulfoxide	Non soluble
3.	Acetone	Non soluble
4.	Methanol, Ethanol, chloroform	Non soluble
5	Hot Water	mucilage
6	Cold water	poorly soluble

3.1.4. Determination of swelling index

Swelling index of powder sample in distilled water was found to be 68 ± 2.125 and shown in **Table 6**. High swelling index indicated that the powder has excellent water uptake capacity. Powder can also interact by means of adhesion with mucous due to swelling by water absorption, and hence can be used as mucoadhesive agent for drug delivery systems.

Table 6: Swelling Index of powder

Method employed	Result
Swelling index	12.7%

Mean ± SD, where n=3

3.1.5. Determination of Melting Point

Melting point of powder sample was determined by capillary fusion method. The melting point was recorded and compared with the literature value and shown in **Table 7.**

Table 7: Melting point of powder

Method employed	Result
Capillary fusion method	134±2.22 ˚C

Mean ± SD, where n=3

3.1.6. Moisture absorption

Moisture absorption of powder sample was determined as per procedure adopted and the results along with literature value are shown in **Table 8**. Hydroscopicity influences the packaging, storage of the products.

Table 8: Moisture absorption of powder

Method employed	Result
Moisture absorption	**8%**

Mean ± SD, where n=3

3.1.7. Loss of drying

The powder sample was subjected for determining the LOD in hot air oven and the inference and comparison with Type value is shown in **Table 9**

Table 9: Loss of drying of powder

Method employed	Result
Loss of drying	**7.5%**

Mean ± SD, where n=3

3.1.8. Determination of pH of powder

The powder sample was subjected for determining the pH in digital pH meter and the inference and comparison with Type1 and Type2 value is shown in **Table 10.**

Table 10: pH of powder

Method employed	Result
Digital pH meter	6.9

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3.2. FLOW PROPERTIES OF BARLEY POWDER

3.2.1. Particle Shape Distribution Particle shape was rough and irregular on microscopic evaluation. The microscopic picture of barley powder shown in **Figure 1**.

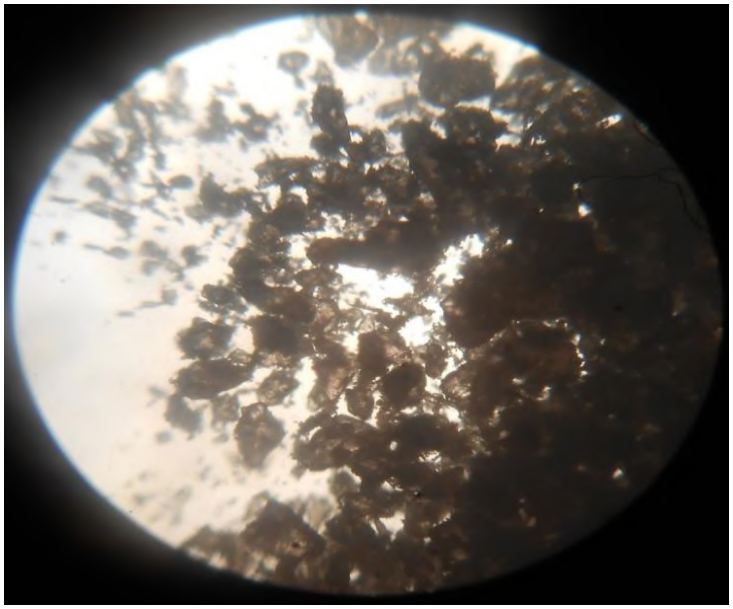

Fig.1: Microscopic study of powder

3.2.2. Micromeritics Properties of barley Powder

The derived properties such as bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose which depend mainly on particle size distribution, particle shape and tendency of the particles to adhere together results shown in **Table 11**.The values of bulk density, compressibility index and Hausner's ratio infer that the barley polysaccharide powder has excellent flow properties and compressibility.

Table 11: Characterization of barley powder

Property	Result
Bulk density (gm/cm^3)	0.72 ± 0.02
Tapped density (gm/cm^3)	0.83 ± 0.03
Compressibility index (%)	15.27
Angle of repose (◦)	29.35
Hausner's Ratio (HR)	1.15

Mean ± SD, where n=3

IV. RESULT AND DISCUSSION

Barley seeds powder was studied as for their identification, characterization and flow properties which is helpful in pharmaceutical industry. Starch,Flavonoids were present in powder and these are insoluble in Acetone, Ethanol and in Chloroform. They were forming the mucilage in hot water but in cold water they were poorly soluble. The pH was to found to 6.9, swelling index 12.7%, Melting point 134^{0}C. The derived properties such as bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose which depend mainly on particle size distribution, particle shape and tendency of the particles to adhere together results shown in Table 9.The values of bulk density, compressibility index and Hausner's ratio infer that the Barley polysaccharide powder has good flow properties and compressibility. The powder sample was subjected for determining the pH in digital pH meter and the inference and comparison with value is shown in Table 8 and found to be 6.9.Particle shape was rough and irregular on microscopic evaluation. The microscopic picture of barley powder shown in Figure 03. According to this study this powder has good flow properties. Barley Powder is medicinally important polysaccharide and it has been reported for the treatment of constipation, inflammation bowl diseases, Stomach cancer, Colon Cancer and high cholesterol.

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DISCLOSURE OF CONFLICT OF INTEREST

All authors declare no conflict interest is exist.

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