World Journal of Pharmaceutical and Life Sciences WJPLS

www.wjpls.org

SJIF Impact Factor: 4.223



Soni Abhishek¹, Thakur Hitesh^{*2}, Dr. Goyal Sachin³, Shivali Singla⁴ and Priyanka Thakur⁵

¹Asst. Prof., School of Pharmacy, Abhilashi University, Chail Chowk, Mandi, H.P.
 ²B.Pharmacy, School of Pharmacy, Abhilashi University, Chail Chowk, Mandi, H.P.
 ³HOD, School of Pharmacy, Abhilashi University.
 ⁴Asst. Prof. School of Pharmacy Abhilashi University.

⁵School of Biological & Environmental Sciences, Shoolini University of Biotechnology & Management Sciences

Bajhol, Solan, H.P.

*Corresponding Author: Thakur Hitesh

B.Pharmacy, School of Pharmacy, Abhilashi University, Chail Chowk, Mandi, H.P.

Article Received on 21/07/2017	
--------------------------------	--

Article Revised on 11/08/2017

Article Accepted on 01/08/2017

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 mucilage from the husk of Plantago ovata and explores its use as pharmaceutical excipients. Powder was isolated by using two different methods. The isolated powder ware investigated for purity by carrying out chemical tests for different Phytochemical constituents and only carbohydrates were found to be present. The powder was further characterized for physical and flow properties. Type 01 Powder has good swelling index 62, _PH 6.6, Melting point 137, Moisture absorption 2.10, Loss on drying 1.70. 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 Plantago ovata's husk powder has satisfactory _PH and physicochemical Properties, which can be used as pharmaceutical excipients in formulating various dosage form.

KEYWORDS: Plantago ovata, Powder, husk, Isolation, Characterization.

1. INTRODUCTION

The origin of the word Plantago ovata lies in the Persian word ISAP which means the horse and Ghol means the ear.^[1] Thus, the literal meaning of word isapgol is the ear of the horse. Plantago ovata was introduced as a medicinal plant by Indian Muslims and seeds were firstly collected from some wild species. In Pakistan, it was firstly cultivated in Lahore and Multan districts and then moved to Bengal, Mysore and Indian Coromandel coast. Plantago ovata (Psyllium) was a native of Persia, now grown in the western part of India.^[3] The term "Psyllium" is used for the crust, seed and the whole plant. It is considered as a good source for soluble and insoluble fiber. Its soluble content is almost eight times more than that of oat's bran.^[5] The diet fibers extracted from the plant possess pharmaceutical properties and can be used in producing low calorie food. The world market is dominated by India in the production and export of Plantago ovate.^[4] The crop is mainly cultivated in Gujarat, Madhya Pradesh and Rajasthan. In India Gujarat and Rajasthan states are the major producer states of Plantago ovata because the climatic conditions of them are most suitable for the Plantago ovata cultivation; and both the states contribute almost equally in terms of production, but regarding further processing and

manufacturing of husk, Gujarat is the leading state contributing 35% of world production of Plantago ovata Husk. The seeds, as well as, husk of the seeds, are used in medicine since 18th century. About 10 species of the drugs are available in India. Seeds are very small in size. A thousand seeds weigh about 1.5g. Isapgol has high export potential value. Other names of Isabgol are Ispaghula, isapgol etc. Isabgol is a rubi crop and needs well – drained loamy soil, cool and dry weather. Heavy rains and cloudly weather at its maturity affect the yield adversely. The drug is cultivated by broadcasting method, in the month of November.^[5]

1.1 Biological Source

Isabgol consists of *Plantago ovata Forskal*, (Family: Plantaginaceae). In the pharmaceutical field, seeds, as well as the dried seeds coats, known as Isapgol husk, are used.^[2]



Fig 1: Plant of Plantago ovate.

1.2 Geographical Distribution

The plant is cultivated largely in Gujarat, Punjab, and South Rajasthan. The factory for preparation of husk is located at Sidhpur in North Gujarat. In Maharashtra, it is found to be grown successfully near Pune, about 30 thousand hectares of area is said to be under cultivation for the drug in India.^[2]

1.3 Organoleptic Characters

Colour: Pinkish – grey or brown. Odour: none Taste: Mucilaginous, bland. Size: 10 to 35 mm in length and 1 to 1.75 mm in width, Shape: It is ovate cymbiform. One thousand seeds weigh about 1.5 g.

1.4 Extra Features

Seeds are hard, transparent and smooth with grey or reddish brown oval spot in the centre of the convex surface. Concave surface contains the hilum covered with thin membrane having two perforations.

1.5 Plantago Ovata Husk

Plantago ovata seeds are processed to take out seeds coats, commercially known as husk. The husk makes up about 25 to 27 % of the seed. The seeds are thoroughly dried and sieved to get rid of foreign organic matter. Seeds are crushed in flat stone grinders by passing several times through them, so as to cause the complete removal of the coating. The crushed material is then winnowed to separate the kernels and husk. Husk is sieved to get different grades and sizes. Thus, it consists of the epidermis and its adjacent layers removed from the dried seeds of *Plantago ovata*. Morphologically, it is in the form of pale buff ovate flakes with more or less lanceolate shape. The pieces are 1 to 2 mm in size.^[3]



Fig 2: Plantago ovate Husk.

1.6 Chemical Constituents

Plantago ovata and seeds contain mucilage which is present in the epidermis of the seed. Chemically, it consists of pentosan and aldobionic acid, the products of hydrolysis are xylose, arabinose, galacturonic acid and rhamnose. Fixed oil and proteins are other important constituents of the drug.^[5]

1.7 Medicinal use of Plantago Ovata^[8,9] a). Loose Motion and Dysentry

In order to get relief from this condition one could take Plantago ovata with curd. Curd or yogurt has got gut repairing properties which helps in this problem if taken with Plantago ovata and gives fast relief from Dysentery. Isabgol (soluble fiber) helps in adding fiber or bulk in the stools while curd adds microbial agents in the stomach which helps in healing the gut. The soluble fibers present absorb intestine's water and swells which in turn binds with the stools and make them bulky.

Method: Mix 3 tea spoon of the husk with 6 tsp of curd and consume it before going to bed and after meal. You could also take it twice a day for fast relief.

b). Help in Constipation

Believe it or not but this is only food which helps in both constipation and diarrhea. It is widely used in Indian subcontinent for this condition due to its laxative properties and rich fiber content. Once in digestive tract it expands and it helps in bowel movement because of gelatin base present in it. The fibers present in it has hygroscopic properties due to which it binds water molecules and helps in proper digestive functions in addition to motility of gut. All these makes passing stools easy without any pain.

Method: Mix 2 tsp of it with warm milk and take it just before going to bed. If the problem is severe then you could add 1 tsp of sugar to the above which would increase its effectiveness. If milk is not available then one could take it with warm water which is also very effective.

c). Help in Acidity

Acidity is one of the major problems of recent times due to wrong type of food we eat (fast food) and less sleep. Plantago ovata husks are one of the safe home remedy for this problem without any side effects. The husk helps protect from irritation by making a protective layer to lining of the stomach. It also reduces the digestive acid by nullifying its effect. It also does helps in reducing the occurrence of hyperacidity episodes by reducing the release of stomach acids.

Method: Mix 1-2 tsp of husk in milk or water and take it immediately when you feel the burning sensation due to acidity problem. Do not take it with warm or hot water/milk as it could have negative effects. The liquid should not be very cold, room temperature might work best.

d). Help in weight loss

Plantago ovata expands in the stomach, it gives a sense of fullness for long period of time which in turn reduces cravings for food. It also cleanses the colon by improving gut motility and removing the wastes from stomach effectively which improves the overall digestive system.

Method: Mix 1-2 tsp of the husk with warm water and half lemon juice just before meal or in the morning. Warm water, lemon with 1 tsp of honey in the morning alone helps. If husk is added to it could help its effectiveness further.

Other uses of Isabgol are as Below

- It is nutritious.
- It helps in getting rid of bad breath.
- Toothache can be treated as well. Mix Isabgol in vinegar and apply it on the painful tooth.
- It helps in treating headache by grinding some eucalyptus leaves and then applying the paste on the forehead.
- One can take 3 to about 5 grams of Isabgol seeds along with hot water in the morning to get rid of much relief from asthma, as well as breathing problems.
- Anal fissures can be cured by it.
- Blood glucose as well as insulin can be regulated by it.

1.8 Precautions

Do not take it without mixing it in adequate amount of water or other fluids. If done so it might block the throat causing choking. It should not be taken if you have any issues while swallowing. Plantago ovata could also cause allergic reactions in few people especially to those who have a regular exposure to Plantago ovata dust.

1.9 Side Effacts of Plantago Ovata

Plantago ovata is widely used in India to get relief from gastrointestinal conditions like constipation, diarrhea and acidity. It is also touted be an effective weight loss tool and is known to keep your heart healthy. Since it is associated with many health benefits, we don't think twice before using it and ignore the fact that using it excessively and in an improper way can have side effects too.

a). Affects absorption of minerals

A study found that consumption of 25 g Plantago ovata husk increases the faecal excretion of trace minerals like zinc, copper and manganese. It also lowered their retention in the body due to which, the serum levels of these trace minerals dropped significantly. These trace minerals are equally important in maintaining good health. Zinc improves immunity, promotes enzyme activity and helps in healing wounds. Manganese prevents the wear and tear of bones and connective tissues. It helps in calcium absorption, blood sugar regulation and fat and carbohydrate metabolism. Copper, on the other hand, is important for production of red blood cells and strengthening the immune system.

b). Can interfere with the absorption of drugs

Plantago ovata tends to adsorb the drug on its surface due to which its absorption in the blood from the gastrointestinal tract or the small intestine reduces. As a result of which, the effectiveness of the drug is reduced. This claim holds true for aspirin, but it is believed that isabgol interferes with the absorption of other drugs too. However, there is no proof to support this claim.

c). Can cause bloating

Plantago ovata is high in fibre and is hence prescribed to people suffering from constipation and other gastrointestinal disorders but consumption of too much fibre affects the passage of gas from the GI tract to the rectum, leading to gas retention that results in bloating.

2. MATERIALS AND METHODS

2.1 Isolation of Powder from Plantago Ovata^[3]

Powder from Plantago ovata husk was prepared by two methods. Powder from method 01 marked as Type 1 and powder from method 02 marked as Type 2.

Method 01

The Plantago ovata husk were soaked in distilled water for 48 hrs. Then boiled for 20 minutes. The collected material was squeezed through muslin cloth to separate them. Then, an equal volume of acetone was added to the filtrate for precipitation of the mucilage. The separated mucilage was dried at 40C in a tray dryer. The dried mucilage was powdered and sieved in sieve no # 80.The resultant powder was stored in a desiccator and used for the present study.



Fig 3: Soaked Plantago ovata husk.

Method 02

The Plantago ovata husk were soaked in distilled water for 48 hrs. Then boiled for 20 minutes. The collected material was dried at 60° C in a tray dryer. Then grind the dried mucilage. The grinded mucilage was powdered.

2.2 Physicochemical Characterization of Selected Plantago Ovata^[3]

Plantago Mucilage was characterized by various tests of identification.

a). Determination of purity of gum

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

b). Identification of Mucilage

Powdered mucilage was treated with ruthenium red solution and observed for the appearance of pink color.

c). 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.

d). Swelling index

Swelling index of mucilage polysaccharide was determined by using modified method reported. One gram of powder (#100 mesh passed) was accurately weighed and transferred to a 100 ml stopper 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 stopper, 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]

e). Solubility

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

f). Melting point

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

g). Moisture Absorption

The mucilage powder was weighed accurately and placed in a desiccator. After 3 days, the mucilage 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.

h). Loss on Drying (LOD)

Moisture content of Isapghula mucilage was determined by loss on drying method. Accurately weighed 1 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

i). pH of Mucilage

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

j). 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 powder showed a change in color was noted.

2.3 Flow Properties of selected Polymers^[3]

a). Determination of particle shape distribution Isapghula mucilage 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. $^{[3]}$

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

Where, ρ_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, ρ_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.

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

Where, θ is Angle of Repose, h is 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

d). 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).

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

Where; I is compressibility index, ρ_t is Tapped Density, ρ_b is Bulk Density. $^{[63]}$

Table 2: Compressibility index.

Carr's index (%)	Type of flow
<12	Excellent
1217	Good
18-21	Fair to passable
22-32	Poor
33-38	Very poor
>40	Extremely poor

Table 3: Organoleptic evaluation of the mucilage powder.

S. No.	Property	Type 1	Type2
1.	Color	Light brown	Light brown
2.	Odor	Odor less	Odor less
3.	Taste	Tasteless	Tasteless
4.	Shape	Irregular	Irregular
5.	Touch and Texture	Hard and Rough	Hard and Rough

c). 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

Table.4. 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	Type1	Type2
1.	Test for steroids: Libermann – burchard test		Absent
2.	Test for saponins : Foam test		Absent
3.	Test for Carbohydrates: Molisch test, Barfoed's test, Benedicts test		Present
4.	Test for Flavonoids: Shinoda test, Zinc/HCl reduction test	Absent	Absent
5.	Test for Tannins/ Phenols: Ferric chloride test, Gelatin test	Absent	Absent

d). Solubility Profile of powder

The solubility profile of the powder was found to as shown in Table 5. The powders were insoluble in acetone, alcohol, ether, chloroform. It was found to form a gel in Hot water.

Hau

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).

3. RESULT AND DISCUSSION

d). Hausner's Ratio

3.1 Physicochemical Characterization of Isapghula a). Identification of Mucilage

Powdered mucilage was treated with ruthenium red dye solution and observed pink color passing the test for mucilage.

b). Organoleptic Evaluation

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

S. No.	Solvents	Type1	Type2
1.	Acetone	Insoluble	Insoluble
2.	Methanol, Ethanol, chloroform	Insoluble	Insoluble
3.	Hot Water	Forming a gel	Forming a gel
4.	Cold water	Poorly soluble	Poorly soluble

Table 5: Solubility behavior of the selected powder.

e). Determination of swelling index

Swelling index of powder sample in distilled water were found to be 62 ± 1.527 and 60 ± 1.52 shown in Table 6. High swelling index indicated that the mucilage has excellent water uptake capacity. Mucilage 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	Type1	Type 2
Swelling index	62 ± 1.527	60±1.52
Mean \pm SD, where n=3		

f) 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	Type 1	Type 2
Capillary fusion method	137± 1.121°C	$136 \pm 1.122^{\circ}C$

Mean \pm SD, where n=3

g). 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. Hygroscopicity influences the packaging, storage of the products.

Table 8: Moisture absorption of powder.

Method employed	Type1	Type2
Moisture absorption	$2.10 \pm 0.7843\%$	2.43 ± 0.5774 %
Mark CD 1	2	[

Mean \pm SD, where n=3

h). Loss of drying

The powder sample was subjected for determining the LOD in hot air oven and the inference and comparison with Type1 & Type2 value is shown in Table 9.

Table 9: Loss of drying of powder.

	Method employed	Type1	Type2	
	Loss of drying	$1.70 \pm 0.4532\%$	$1.61 \pm 0.3606\%$	
1	Mean + SD where $n=3$			

Mean \pm SD, where n=3

i). 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	Type1	Type2
Digital pH meter	$6.6 \pm 0.34\%$	6.4±0.48%

j). Thermal Stability

Thermal stability study was established and the mucilage withstands to temperature up to Type1 =140°C and Type 2 = 135 °C showing high thermal stability.

4. Flow Properties of Isapghula Powder

a). Particle Shape Distribution Particle shape was rough and irregular on microscopic evaluation. The microscopic picture of Isapghula powder shown in Figure 03.

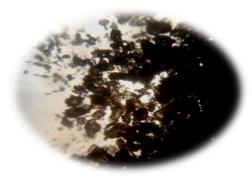


Figure 3: Microscopic study of powder.

b). Micromeretics Properties of Isapphula 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 Isapphula polysaccharide powder has excellent flow properties and compressibility.

Property	Type1	Type2
Bulk density (gm/cm ³)	0.72 ± 0.002	0.71 ± 0.002
Tapped density (gm/cm ³)	0.83 ± 0.001	0.80 ± 0.001
Compressibility index (%)	15.27 ± 0.003	12.67 ± 0.003
Angle of repose (°)	29.35 ± 0.004	31.02 ± 0.004
Hausner's Ratio (HR)	1.15 ± 0.002	1.12 ± 0.002

Table 11: Characterization of Isapphula powder.

Mean \pm SD, where n=3

5. RESULT AND DISCUSSION

Isabgol husk powder was prepared by two methods as Type-1 and Type -2. These powders were studied as for their identification, characterization and flow properties which is helpful in pharmaceutical industry. Carbohydrates, Alkaloids and fixed oil was present in both Type-1 and Type-2 powder. These are insoluble in Acetone, Ethanol and in Chloroform. They were forming the gel in hot water but in cold water they were poorly soluble. The pH was to found to 6.6 and 6.4 and 6.4, swelling index 62 and 60, Melting point 137 and 136, Moisture absorption 2.10 and 2.43,Loss on drying 1.70 and 1.61. The bulk density was found to 0.72 and 0.71, Tapped density was found to 0.83 and 0.80, angle of repose was found to be 29.35 and 33.02 and the compressibility index was found to 15.27 and 12.67, Hausnor's ratio was found to be 1.15 and 1.12 respectively. According to this study this powder has good flow properties. The swelling index was found to 62 and 60. The melting point was found to 137 and 136. Isabgol husk is medicinally important polysaccharide and it has been reported for the treatment of constipation, diabetes. diarrhea, inflammation bowl diseases. ulcerative colitis, cancer, obesity, high cholesterol, and so forth.

6. ACKNOWLEDGMENT

I am thankful to Mr. Abhishek Soni, Assistant Professor, School of Pharmacy, Abhilashi University, Chail Chowk, who guide and motivate me to do research work in my first year of B.Pharmacy.

7. REFERENCE

- 1. www.wikipedia.com/isabgol.
- 2. Kokate K.C., Purohit P. A.,and Gokhale.S. B.: Text book of phamacognosy, published by Nirali Prakash an, Edition, 4: 9.9 9.12.
- Soni Abhishek, Raju.L," Formulation And Evaluation of Fast Disintegrating Tablet Containing Hydrochlorothizide" Indian Journal of Pharmacy and Pharmacology, April-June 2015; 2(2): 119-133.
- Baveja SK, Gupta BM. Rheology of Aqueous dispersions of Plantago ovata seed husk-I. Indian J Pharm Sci, 1968; 30: 187-94.
- Fischer, H.M., Nanxiong, Y., Ralph, R.G.J., Andersond, L. and Marletta, J.A. The gelforming polysaccharide of psyllium husk (Plantago ovata Forsk). Carbohydrate Research, 2004; 339: 2009– 2017.

- Ganji, V. and Kuo, J. Serum lipid responses to psyllium fiber: differences between pre- and postmenopausal, hypercholesterolemic women. Nutrition Journal, 2008; 7: 22. doi:10.1186/1475-2891-7-22.
- Garg, P., Raghav, P.K., Sharma, R.K., Jasuja, N.D., Sharma, R., and Agarwal, N. Development of Mucilaginous Spongy Dessert-A Herbal Rassogolla Prepared from Cow Milk. International J. of Scientific and Research Publications, 2014; 4(2): ISSN 22503153.
- Majmudar, H.; Mourya, V.; Devdhe, S. and Chandak, R. Pharmaceutical Applications of Ispaghula Husk: Mucilage. International J. of Pharmaceutical Sciences Review & Research, 2002; 18(1): 49-55.
- Mironeasa, S., Codină, G.G. and Popa, C. Effect of the addition of Psyllium fiber on wheat flour dough rheological properties. Recent Researches in Medicine, Biology and Bioscience, 2013; ISBN: 978-960-474-326-1.