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RESEARCH ON PHYSICOCHEMICAL CHARACTERIZATION OF MUCILAGE FROM THE SEEDS OF *PYRUSCYDONIA*

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ABSTRACT

The present research was related to a process of production of pharmaceutically useful dry water-soluble mucilage/polysaccharide from the seeds of *Pyrus Cydonia*. Extraction of water-soluble mucilage from the seeds of *Pyrus Cydonia* was performed using hot water, ethanol and acetone as a solvents for better yield. Physicochemical characterization and other studies such as viscosity, pH, swelling Index, solubility, film-forming capacity, and loss on drying were discussed. The percentage yield of mucilage was found 10%. It was found that mucilage formed a colloidal solution in cold & hot water. A viscosity study of mucilage was done by using Brookfield viscometer. The *Pyrus Cydonia* mucilage formed a fragile film after it has been incubated at 50°c for 24 hr. When it was exposed to the air, the film became sticky, flexible and stretchable. The swelling index of mucilage was found in a different solvent such as distilled water, gastric fluid 0.1N Hydrochloric acid and it was found 23.39±0.061 % and 13.29±0.06 % (vol/vol) respectively. The pH of 1% w/v solution of mucilage in water was 5.6 and at 0.1N HCl was 2.12, which indicated that the product was acidic in nature. The Powder characterization study such as bulk density and tapped density of mucilage were 0.45, 0.62 respectively and Carr's index and Hauser's ratio was 32.25 and 0.72±0.05. The moisture content of mucilage was 1%. The angle of repose was 38·40°. Scanning electron microscopy was indicated an uneven size structure. The FTIR spectrum shows peaks at 3377.83 cm^{1} for *Pyrus Cydonia* indicating presence of hydrogen bond and 1245.25 cm^{-1} indicated for alkyl group. Biological evaluation of dried mucilage was carried out by determination of LD50 of dried mucilage of *Pyrus Cydonia* and it was found safe in mice after its administration by the oral route. Hence, the extracted dry water-soluble mucilage/polysaccharide from *Pyrus Cydonia* may be useful in the field of pharmacy, because it was found economic, non-toxic and biocompatible in nature.

Keywords: Mucilage / polysaccharide, viscosity, swelling Index, film forming.

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INTRODUCTION

Mucilage is most commonly used adjuvant in pharmaceutical preparations. Plant mucilage are pharmaceutically important polysaccharide with wide range of applications such as thickening gelling agent, binding, disintegrating, suspending, emulsifying, stabilizing and gelling agents. They have been also used as matrices for sustained and controlled release drugs. Naturally available mucilage is preferred to synthetic materials due to their non toxicity, low cost, emollient and non irritating nature[1]. Acacia, tragacanth, gumghati, gum Karaya are popular examples of plant mucilage. As a dose formulators essential to develop cost-effective and less tedious procedures for preparation of sustained release formulations on the industrial scale. The most commonly used method for fabricating drugs in a controlled-release formulation is by incorporating them into a matrix containing a hydrophilic rate controlling natural polymer[2].

The present research was related to a process of production of pharmaceutically useful dry water-soluble mucilage/polysaccharide from the seeds of *Pyrus Cydonia*. Extraction of water-soluble mucilage from the seeds of *Pyrus Cydonia* was performed using hot water, ethanol and acetone as a solvents for better yield. Physicochemical characterization and other studies such as viscosity, pH, swelling Index, solubility, film-forming capacity, and loss on drying were discussed.

MATERIAL AND METHODS

The plant materials were purchased from local market of Pusad, Asadi Ayrvedic & Kirana stored, Gandhi chock, Pusad. Dist: Yavtmal. Plants used for the present study Viz *Pyrus Cydonia* was identified by the Department of Botany at Phulsing Naik College, Pusad, Dist. Yavatmal. Aspirin was purchased from Alkem Pharmaceuticals. Hydroxyl propyl methyl cellulose, Ethanol was purchased from Zim laborties. The other chemical and solvents used were Analytical grade.

Preparation of mucilage by boiling hot method:

Mucilage was extracted by heating with hot water. Extraction was carried by electric stirring of the mixture of distilled water and seeds with a ratio of 1:40 (w/v). The extract was left to room temperature. The extract was then centrifuged at 4500xg for 30min at 5^0c and then filtered through glass wool. The mucilage was precipitated from the extract by adding ethanol. After allowing stand for 24h at 5^0c, the precipitated was removed by centrifugation at 4500xg for 30 min at 5^0c. Final precipitated was homogenized in little water & adjusted to pH 7 by 0.5M Sodium hydroxide. The concentrate was freeze dried in a freeze dryer (Va CO5, Zirbus technology Osterode (Germany). The freeze dried mucilage powder was stored in closed container.[3]

Physical Property of mucilage:

Organoleptic Evaluation of Isolated mucilage:

The isolated mucilage was characterized for organoleptic properties such as color, odor, taste, fracture and texture.

Chemical property:

Identification tests for carbohydrates, proteins, mucilage

Aqueous solution of extracted mucilage was used for chemical characterization. Test for carbohydrates, proteins, mucilage, alkaloids, fats, tannins amino acids and gums were performed according to standard procedure.

Physicochemical Characterization of Isolated Mucilage:

Percent yield:

The percentage yield was calculated on the basis of amount of seeds of *Pyrus Cydonia* species used for the extraction process and amount dry water soluble mucilage/polysaccharide obtained individually depends upon solvent used. The percentage calculated using the formula mentioned below.[4]

$$\%yeild = \frac{Wt.ofdried\ mucilage\ obtained}{Wt.ofseeds\ taken} \times 100$$

Solubility Behavior mucilage:

One part of dry mucilage powder was shaken with different solvents and the solubility of dry mucilage powder was determined.

Viscosity of mucilage

Viscosity of *Pyrus Cydonia* mucilage was determined by preparing 2% w/v suspension in double distilled water. The dried powdered mucilage's was suspended in double distilled water for 2 hours to prepare a uniform suspension. The intrinsic viscosity of mucilage was measured using spindle number 62 at 30.4^0C at torque of 34.4%. Whereas, for the measurement of intrinsic viscosity of mucilage required spindle number 61 at 30.4^0C at torque of 15.9%. Viscosity was determined by using a Brookfield viscometer.[5]

Film forming

Film forming capability was performed by spreading 6.7% aqueous mucilage (1g dried *Pyrus Cydonia* mucilage in 15ml water) on to the petriplate and incubated at 50^0c for one day.[6]

Swelling Index of Isolated mucilage:

The swelling index is the volume (in ml) taken up by the swelling of 1 g of test material under specified condition. The swelling index of the mucilage was determined by accurately weighing 1g of mucilage, which was further introduced into a 25ml glass-Stoppard measuring cylinder. 25ml of water was added and mixture was shaken thoroughly every 10 min for 1 h. It was then allowed to stand for 3h at room temperature. Then the volume occupied by mucilage, were evaluated for different technological parameters such as hardness, friability, thickness, diameter, drug content and *in vitro* drug release according to official guidelines[7].

pH of mucilage:

pH fundamentally represents the value of hydrogen ion activity in solutions. pH was determined by preparing 1% w/v of suspension in freshly prepared double distilled water. The dried powdered mucilage's was suspended in double distilled water for 4 hours. The solution was homogenized by mechanical stirrer for 1 hour. pH of the resultant solution was measured using electronic digital pH meter model EQ 610 made of Equiptronics.

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Bulk Density (Bd) and Bulkiness:
The inverse of bulk density is called as bulkiness. Accurately weighed quantity of (50g) was introduced into a graduated measuring cylinder. The cylinder was fixed on the bulk density apparatus and the volume occupied by the powder was noted. Then, the powder was subjected to tapping in a bulk density apparatus until constant volume was obtained. The final volume (bulk volume) was noted. [8]

Loss on drying:
The loss on drying of *Pyrus Cydonia* mucilage was determined using a modification of the procedure reported by Mazza and Biliaderis (1989).The mucilage was dispersed in distilled water (0.5%w/v) and resultant slurry heated under stirring at the desired temperature for 30 min in a temperature controlled water bath cooled at 10°c and centrifuged at 10°c for 10min at 5000 rpm and 10 min at 10,000 rpm. The aliquots of supernatant were dried to determine the percent solubilisation of dried mucilage[9].

Tapped density (Td)
The tapped density or poured density attained after mechanically tapping a container containing the powder sample. The standard method described in USP was followed and tapped density was calculated using equation given below:
Td = M/Vp
Where,
M = weight of samples in grams and
Vp= final tapped volume of powder in cm^3.

Carr's index
An indirect method of measuring powder flow from bulk densities was developed by Carr. A low Carr's index implies a good initial packing arrangement, with less volume of voids. As the value of these indices increases, the flow of the powder decreases. In general, however, Carr's index below 16% indicates good flow ability while values above 35% indicate cohesiveness.
Carr's index of each sample was calculated according to equation given below-
CI =100 (Td-Bd/Td)

Hausner's ratio
Hausner's ratio measures the powder ability to settle and permit an assessment of the relative importance off end particulate interactions. Hausner's ratio is calculated as the ratio of bulk density to tapped density. The Hauser's ratio less than 1.25 indicates good flow; the values between 1.25 to 1.5 assures that adding glidant will improve flow ability.[9]
HR= V0/Vf

Where,
V0: unsettled apparent volume,
Vf: final tapped volume

Moisture content determination
The prepared dried powder mucilage's are hydrophilic in nature. They have capacity to absorb and retain moisture. Hence, it is necessary to determine moisture content of prepared dried mucilage. The moisture content is the ratio, expressed as a percentage, of the mass of "pore" or "free" water in a given mass of powder to the mass of the dry solids. *Pyrus Cydonia* mucilage was subjected to moisture content test as per the following method. One gram (1g) of powder was weighed and then dried in an oven at 105°C for about 1 hour and then weighed again until constant weight was attained and the % Moisture content was determined.[5]
Wf/wi×100
Where;
Wf is final weight of powder after drying
Wi is initial weight of powder before drying.

Angle of Repose
Angle of repose has been defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the granules of each formulation was determined by the fixed height funnel method. The angle of repose was calculated by substituting the values of the base radius 'r' and pile height 'h' in the following equation.[8]
$\tan \Theta = h/r$
Therefore; $\Theta = \tan^{-1} (h/r)$.

Scanning electron microscopy (SEM)
SEM of *Pyrus Cydonia* mucilage carried out by Diya Labs, c/o Ambika medical, shop no3, Airuvalinavi, Mumbai 400708.

Fourier Transform Infrared (FTIR) spectroscopy:
FTIR spectra were recorded by mixing dried mucilage powder with potassium bromide (KBr) of IR grade using Aligentcary 630 ATR, FTIR-4100 model. Pellets were prepared with KBr by means of hydraulic press at a pressure of 50 units. The scanning range was 4000-400cm^{-1}.[5]

Biological Evaluation:
LD-50 determination:
This is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animal when administered by the oral route. This is the LD50 value is expressed in term of weight of test substance per unit weight of test animal (mg/kg). As per OECD guideline No.425, the experiment was started at dose 5000 mg/kg b.w. by down method. In these requirement animal is less then up dosing method.

Characterization of mucoadhesive property of the mucilage:
Mucoadhesive drug delivery systems were developed to sustain drug delivery via various mucus membranes for either local or systemic delivery of poorly absorbed drugs such as peptides and proteins.[10-11]. Most of the mucoadhesive materials are either synthetic or natural hydrophilic or water-insoluble polymers and are capable of forming numerous hydrogen bonds because of the presence of carboxyl, sulfate, hydroxyl, and amino functional group. Formation of hydrogen bonds among the functional groups of the polymers and mucosal layer plays an important role. In general, stronger the hydrogen bonding stronger is the adhesion. Various polymers which have the ability to form strong hydrogen bonds include poly (vinyl alcohol), acrylic derivates, celluloses, and starch.[12] Apart from the hydrogen bond

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formation, the presence of functional groups within the polymer structure may render the polymer chains as poly electrolytes. The presence of charged functional groups in the polymer chain has a marked effect on the strength of the bio adhesion and can be demonstrated by the cell-culture-fluorescent probe technique.[13]

Method of Determination

Mucoadhesive property of *Pyrus Cydonia* mucilage, Hydroxyl Propyl Methyl cellulose, starch was determined according to Park and Robinson method. The Extracted mucilage was glued on to the lower platform of the equipment for Mucoadhesive determination. An excised sheep duodenum measuring 2cm width by 3cm length was attached to arm of the equipment by mean of glue.

The mucosa was gently brought in to contact with the moistened disc and adhesion was allowed to take place for 5, 10, 15, 20, 25 minutes. At the end of time intervals, the mucosa was gently detached from the mucilage disc and the force was directly recorded depending occurred on an electronic balance. The mean of three determinations was obtained[14]. Mucoadhesive property of mucilage, HPMC and starch were shown in Fig 1.

RESULTS AND DISCUSSION

Mucilage was extracted by boiling hot method. The mucilage comprises of yellowish brown and odorless, which was hydrated rapidly on contact with water to swell, but soluble in cold water and hot water and formed a colloidal suspension.

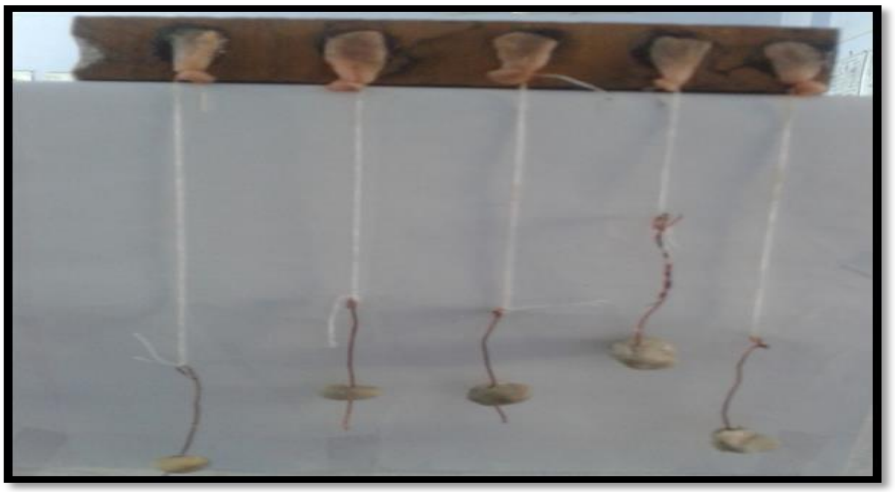

Figure 1: Mucoadhesive strength determination

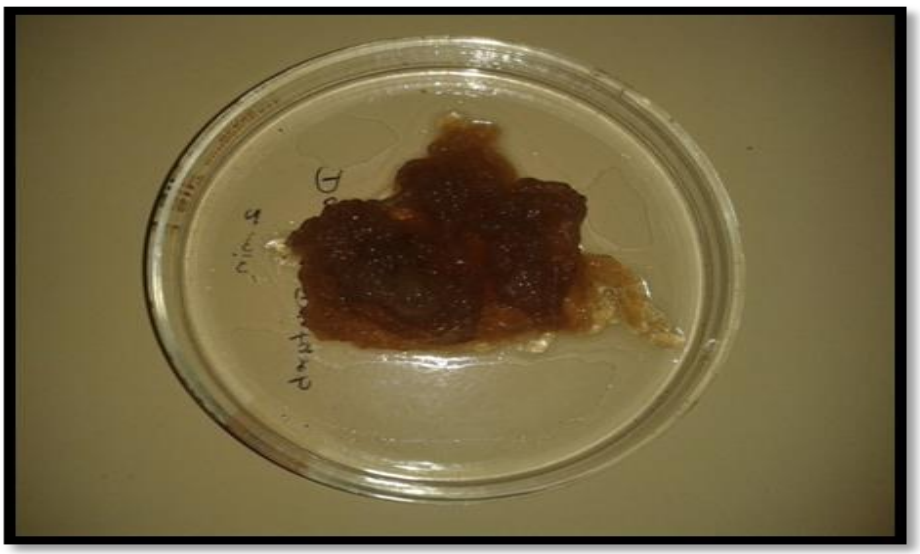

Figure 2: Isolated Mucilage from seed of *Pyrus Cydonia*

Results of organoleptic properties such as colour were brownish, odour and taste was none which was tabulated in table 1.

In table no 1. Photochemical investigation of isolated mucilage showed the presence of mucilage, carbohydrates while gum, tannins, alkaloids and proteins, aminoacid, starch showed negative test. Results obtained after phyto chemical test mention in table no 2.

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Table 1: Organoleptics properties of mucilage

Colour	Odor	Taste	Nature	Texture
Brownish	None	None	Powder	Smooth

Table 2: Chemical test (+Present; -Absent)

Tests	Present/Absent
Mucilage (Ruthenium red).	**+**
Proteins	**-**
Fats and oils	**-**
Tannins	**-**
Carbohydrate (Molishs test)	**+**
Amino acids	**-**
Starch	**-**
Terpinoids	**-**
Alkaloids	**-**

The percentage yield of mucilage was found 10%. Extract formed a colloidal solution in cold and hot water. Viscosity study of mucilage was done using Brookfield viscometer. Viscosity of 2% W/V solution of mucilage was found 40cps. The *Pyrus Cydonia* mucilage formed fragile film after it has been incubated at 50^{o}c for 24 hr. When exposed to the air, the film becomes sticky, flexible and stretchable. The swelling index of mucilage was found in different solvent such as distilled water. Gastric fluid 0.1N Hydrochloric acid was found to be 23.39±0.061% and 13.29±0.06 % (vol/vol) respectively. The pH of 1% W/V solution of mucilage in water was 5.6 and and at 0.1N HCl was 2.12, which indicated that the product was acidic in nature (Table No. 3).

The dried powdered mucilage of *Pyrus Cydonia* was subjected for bulk density, tapped density, Carr's index and Hauser's ratio (Table 5). Bulk density and tapped density of mucilage was 0.45, 0.62 respectively and Carr's index and Hauser's ratio was 32.25 and 0.72±0.05 respectively. The Carr's Index and Hauser's Ratio was measured for the porosity of a powder to be compressed. As such, they measure the powder ability to settle and they permit an assessment of the relative importance of inter particulate interactions. In a free flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter particulate interactions, and a greater difference between the bulk and tapped densities will be observed. Hence, from the data depicted in revealed that mucilage was better flow property based on its bulk density and tapped density. Carr's index was calculated according to the values prescribed in pharmacopoeia.

The moisture sorption capacity is a measure of the moisture sensitivity of the materials. The moisture content may be an extremely important index used for establishing the relationship between the powder behaviour and its properties. The consistency of a fine-grained powder largely depends on its water content. Moisture content of mucilage was 1%. The angle of repose of dried powder mucilage was found to be 38^{0}40 and all parameters are given in table no.3

Table 3: Physico chemical properties of mucilage

Parameter	*Pyrus Cydonia*
Percent yield	10%
Solubility	Soluble in cold water & hot water and form a colloidal solution
Viscosity	40cps
Swelling index	In cold water 23.39±0.06 and 0.1HCl 3.29±0.06
pH (1% w/v solution)	In cold water 5.6 and 0.1HCl .12
Loss on drying % w/w	0.6%
Bulk Density	0.45±0.001
Tapped density	0.62±0.011
Carr's index	32.25
Hausner's ratio	0.72.±0.05
Moisture content	1%
Angle of Repose	38^{0}

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Pyrus Cydonia seed mucilage is a mixture of acidic to neutral polysaccharide which is present entirely in the seed coat. The mucilage layer is being on the outermost (epidermis) of the seed coat. The presence of mucilage in the outermost cells of the seed coat was readily apparent from scanning electron microscope (Fig. 3). Figure shows the fragment of mucilage of uneven size. The cells were filled with the mucilage and cellular structure of cell was completely masked with mucilage, which was shown in fig.3.

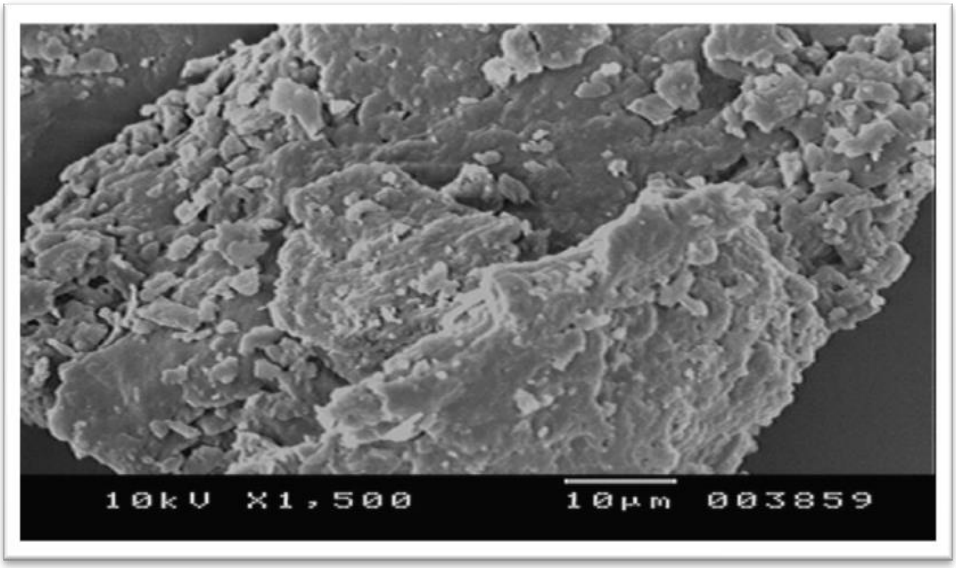

Figure 3: Scanning Electron Microscopy of *Pyrus Cydonia* mucilage

Dried mucilage was collected from *Pyrus Cydonia* plants and it was mixed with KBr to prepare the pellets for FTIR studies.

The FTIR Spectra is shown in fig. 4. It was concluded that the mucilage was polysaccharide in nature. The FTIR spectrum shows a peak at 3377.83cm^{-1}, indicating hydrogen bond and peak at 1245.25 cm^{-1} indicated alkyl group. The above mentioned spectra values can be correlated to the structure of mucilage as a polysaccharide.

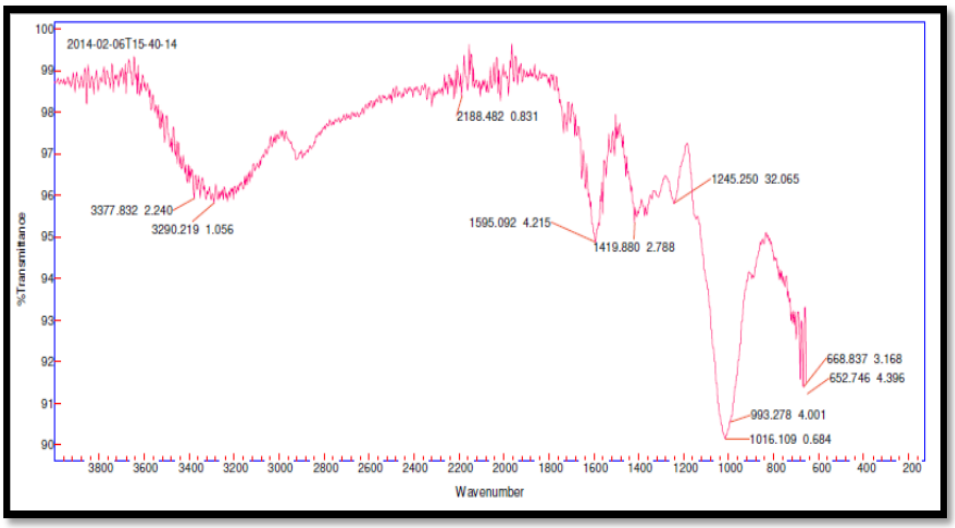

Figure 4: FTIR spectra of *Pyrus Cydonia*.

Biological evaluation of *Pyrus Cydonia* mucilage carried out by LD50 determination. 5000 mg/kg dose of dried mucilage of *Pyrus Cydonia* found to show safe in mice after its administration by oral route. Mucoadhesive property of *Pyrus Cydonia* mucilage, hydroxyl propyl methyl cellulose, starch was determined according to Park and Robinson method. The result showed that the extracted mucilage exhibited higher adhesion and better mucoadhesive property in comparison to HPMC and starch. As a comparison dried *Pyrus Cydonia* mucilage and starch was showed adhesion property and HPMC showed higher mucoadhesive property than *Pyrus Cydonia* mucilage and starch as shown in fig .no 5.

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Table 4: Comparison of Mucoadhesive property of mucilage, HPMC and starch

Sr.no	Time	*Pyrus Cydonia* mucilage (S.D.)	Hydroxyl propyl methylcellusoe	Starch
1	5min	0.370±0.07	0.924±0.08	0.392±0.01
2	10min	0.695±0.09	1.231±0.08	0.738±0.01
3	15min	0.722±0.09	1.615±0.05	0.783±0.01
4	20min	0.994±0.06	2.532±0.05	1.097±0.07
5	25min	1.55±0.04	3.205±0.04	1.595±0.08

***All the values are represents as Mean ± S. D. (n=3)**

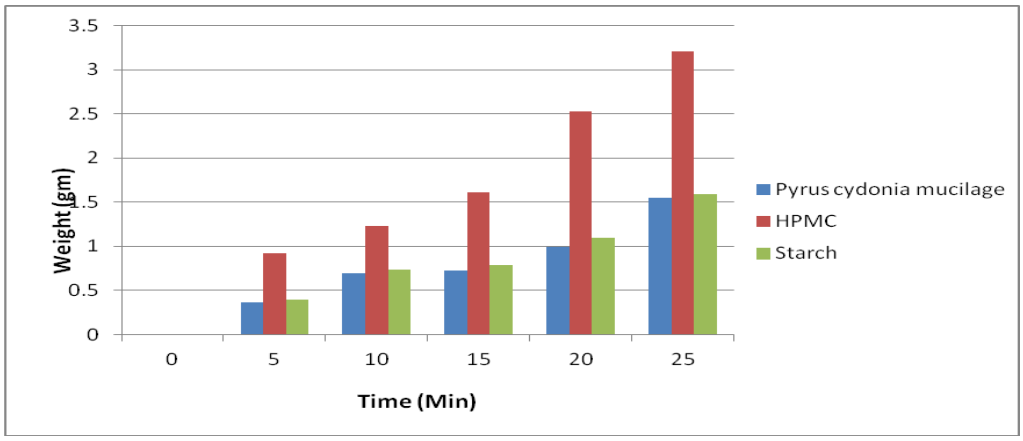

Figure 5: Mucoadhesive strength of *Pyru scydonia* mucilage, HPMC, Starch.

CONCLUSION

The extracted dry water soluble mucilage /polysaccharide from *Pyrus Cydonia* would be economic, nontoxic and biocompatible and it will be useful as a valuable product in pharmaceutical field.

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