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ISOLATION AND EVALUATION OF MUCILAGE FROM CACTUS CLADODES AS A PHARMACEUTICAL EXCIPIENT

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Keywords:

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ABSTRACT

Nowadays, plant derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents , binders, disintegrants in tablets, thickeners in oral formulation, gelliing agents in gels and bases in suppository. These polymers such as natural gums and mucilage are biocompatible, cheap and easily available and are preferred to semi synthetic and synthetic excipients because of their lack of toxicity, low cost, irritant nature. Present study was focusing on isolation of mucilage from herbal plants with their evaluation as pharmaceutical excipient. The mucilage of plant was collected from Cactus cladodes. Characterized for various parameters such as Color, odour, pH, Physical Appearance, solubility etc. Mucilage has been reported to have the property to sustain the drug release, suspending agent, gelling agent. The physical, chemical and microscopical test shows presence and confirmation of mucilage. Study of isolated mucilage shows, it is greenish yellow powder, with sweet odour. It swells in hot water and slightly soluble in cold water and 0.1 N Hydrochloric acid. The bulk density and tapped density of mucilage was found to be 0.49125 gm/ml and 0.60461gm/ml. By analytical investigation like IR spectra and fluorescence analysis mucilage was evaluated and the obtained results shows that the isolated mucilage is acceptable as an pharmaceutical excipient.

1.INTRODUCTION

In recent years, plant derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binders, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository; they are also used in cosmetics, textiles, paints and paper-making.

The plant based polymers have been studied various research group for their application in different pharmaceutical dosage forms like matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions, implants and their efficacy have been reported. These have also been utilized as viscosity enhancers, stabilizers, disintegrants, solubilisers, emulsifiers, suspending agents, gelling agents and bioadhesives, binders in the above mentioned dosage forms.

By the term "mucilage in plants" is meant those substances which are soluble or at least swell very perceptibly in water and which, upon the addition of alcohol, are precipitated in a more or less amorphous or granular mass. Mucilage originates in the plant either as a part of the contents of the cell or as a part of the wall thereof.

Many natural polymeric materials have been successfully used in sustained-release tablets. These materials include: guar gum, isapghula husk, galactomannon from *Mimosa scabrella*, *Gleditsia triacanthos Linn* (honey locust gum), *Sesbania* gum, mucilage from the pods of *Hibiscus esculenta*, tamarind seed gum, gum copal and gum dammar, agar, konjac, chitosan etc. Industrial gums and mucilage, which, for the most part, are water-soluble polysaccharides, have enormously large and broad applications in both food and non-food industries. Their use depends in the unique physicochemical properties that they provide, often at costs below those of synthetic polymers. An understanding of the physico-chemical properties and structural characterization of mucilage is essential in exploiting its potential as a food additive and for other industrial applications.[1,2]

1.1 Mucilage:

Mucilages are heterogenous in composition and are typically polysaccharide complexes formed from the sugars, arabinose ,galactose, glucose, mannose, xylose and uronic acid units. Several plant species such as *Aloe vera, Ceratonia siliqua, Opuntia ficus indica,Basella alba* and *Lepidium sativum* possess mucilage. Mucilages are noted to assume a multitude of physiological functions in plants and act primarily as energy reserves in the rhizomes, roots and seed

endosperms. Foliar mucilages are reported to play a major role in wound responses, plant host pathogen interactions, water transport and responses to a biotic stresses. Extracellular mucilages have been demonstrated to buffer leaf water status against environmental fluctuations and can also enable leaves to maintain low water potential when soil water deficits develop by acting as an apoplastic capacitor. Natural gums and mucilages possess a variety of pharmaceutical properties, which make them useful as additives in pharmaceutical preparations and nowadays, mucilages form vital components in such formulations. Among the different pharmaceutical properties, the suspension property finds application in the preparation of most pharmaceutical suspensions. The suspending agent actually reduces the rate of settling and permits easy re dispersion of any settled particulate matter such as the associated drug. Natural mucilages of *Acacia, Tragacanth, Khaya, Karaya* and *Cassia tora* are being used as suspending agents. There are also reports about the successful use of *Ocimum gratissimum, Butea monosperma, Albiziazygia* gum and *Laucaena leucocephala* seed gum as suspending agents.The natural plant based materials are considered advantageous compared to synthetic polymers because of their natural origin, bio-acceptance, edible nature, renewable nature, environment-friendly processing, low cost and local availability. With the increase in demand for natural mucilages, it has become necessary to explore novel and better sources of mucilage to meet the demands.[6,7]

1.2 Properties of mucilage:

- Mucilages are generally normal products of metabolism, formed within the cell(intracellular formation) and/or are produced without injury to the plant.
- Mucilage are slimy masses.
- They are physiological products.
- Often found in different parts of plant for example in epidermal cells of leaves (senna),in seed caots (marshmallow),barks ,middle lamella (aloe).
- Hydrocolloidal in nature.
- They are also translucent amorphous substances and polymers of a monosaccharide or mixed monosaccharides and many of them are combined with uronic acids.
- Mucilages on hydrolysis yield a mixture of sugars and uronic acids.
- It contains hydrophilic molecules which can combine with water to form viscous solutions or gels.

- Linear polysaccharides occupy more space and are more viscous than highly branched compounds of the same molecular weight.
- The branched compounds form gels more easily and are more stable because extensive interaction along the chains is not possible.[7]

1.3 Advantages of natural mucilage in pharmaceutical sciences:

- **Biodegradable**—Naturally available biodegradable polymers are produced by all living organisms. They represent truly renewable source and they have no adverse impact on humans or environmental health (*e.g.*, skin and eye irritation).
- **Biocompatible and non-toxic**—Chemically all of the plant materials are carbohydrates composed of sugar (monosaccharides) units , they are nontoxic.
- **Low cost**—it is always cheaper to use natural sources. The production cost is also much lower compared with that for synthetic material. India and many developing countries are dependent on agriculture.
- **Environmental-friendly processing**— Mucilages from different sources are easily collected in different seasons in quantities due to simple production processes involved.
- **Local availability (especially in developing countries)** —In developing countries,governments promote the production of plant like guar gum and tragacanth because of the wide applications in a variety of industries.
- **Better patient tolerance as well as public acceptance**— There is less chance of side and adverse effects with natural materials compared with synthetic one. For example Poly metha methacrylate, povidone.
- **Edible sources**—Most mucilages are obtained from edible sources.[7,8]

1.4 Disadvantages of natural mucilage in pharmaceutical sciences:

- **Microbial contamination**—The equilibrium moisture content present in the mucilages is normally 10% or more and, structurally, they are carbohydrates and, during production, they are exposed to the external environment and, so there is a chance of microbial contamination. However, this can be prevented by proper handling and the use of preservatives.
- **Batch to batch variation**—Synthetic manufacturing is a controlled procedure with fixed quantities of ingredients, while the production of mucilages is dependent on environmental and seasonal factors.

- **Uncontrolled rate of hydration**—Due to differences in the collection of natural materials at different times, as well as differences in region, species, and climate conditions the percentage of chemical constituents present in a given material may vary. There is a need to develop suitable monographs on available mucilages.
- **Reduced viscosity on storage**—Normally, when mucilages come into contact with water there is an increase in the viscosity of the formulations. Due to the complex nature of mucilages (monosaccharides to polysaccharides and their derivatives), it has been found that after storage there is reduced in viscosity.[7,8]

1.5 Plant Description

1.5.1 Botanical Name - ***Opuntia cochenillifera*** DC **Family**- Cactaceae

Synonym: Nopal species[2]

1.5.2 Chemical constituents:

Sugar-glucose and fructose ,Vitamins, Polysaccharide- arabinan , Aminoacids, Phenolics (colorless), Betains, Pectins ,Minerals-Calcium,Magnesium ,Potassium, Myricetin, Taxofolin, Rutin, Orientin, Vitexin, Luteolin.[4]

1.5.3 Medicinal uses:

- Used in treatment of metabolic syndrome.
- Used in renal diseases.
- Inflammatory diseases.
- As an anticancer.
- Neuronal disease .
- Pathological process associated with neuronal disease.
- Chronic alcoholism.[3]

1.5.4 Pharmaceutical uses:

- Polymer in sustained release dosage form.
- Gelling agent.
- Suspending agent.[2]

2.MATERIALS AND METHODS

2.1 Isolation of mucilage

The mucilage of plant from the Cactaceae family was collected from Cactus cladodes. Leaves of the plant were collected and diced into small pieces.Diced material was oven dried at temperature of 105 □ c for six hours.Pigments and chlorophyll was removed by treating diced material with pet ether and Chloroform.Obtained material was dried and ground to obtain fine powder.40gram powder was weighed and dissolved in a beaker containing 400 ml water.Powder was allowed to soak of 24 hours.After 24 hours the soaked powder with water was allowed to boil for half hour and then Cooled.Above material was squeezed from muslin cloth and filtrate was collected in beaker.To the filtrate 190 ml of acetone was added and precipitate was obtained.Precipitate was collected in Petridis and dried in hot air oven at 40 □ c. After drying material was collected and ground to obtain fine powder of mucilage.Obtained mucilage was stored in desiccators to protect it from moisture.[8]

2.2 EVALUATION OF MUCILAGE:

2.2.1Microscopic evaluation:

- Mucilage powder was treated with sodalime solution and mounted on glass slide.
- Glass slide mounted with powder was observed under microscope.
- Further the powder was observed using staining agents such as :Sudan red,Acetic acid.
- Mucilage powder was stained and observed under electronic microscope .
- Microscopic image observed was captured using camera attached to microscope.[9]

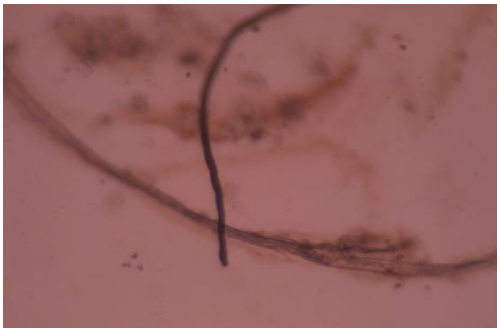

Figure no -1 Trichomes

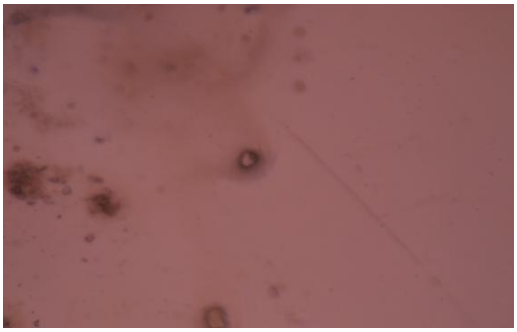

Figure no -2 Starch grains

Powder +Sudan red → Mucilage

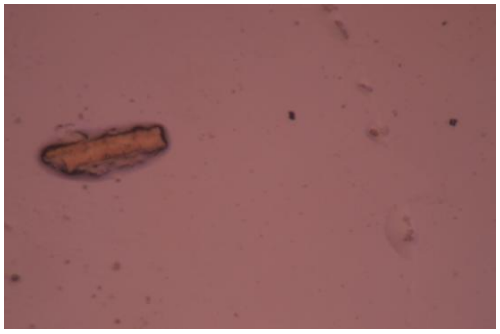

Figure no – 3 Vascular Bundles

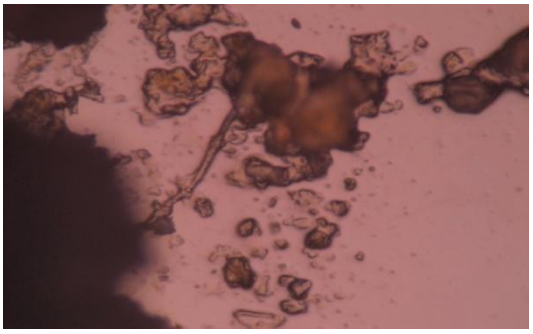

Figure no - 4 Mucilage

Powder + acetic acid ⟶ Calcium oxalate crystals

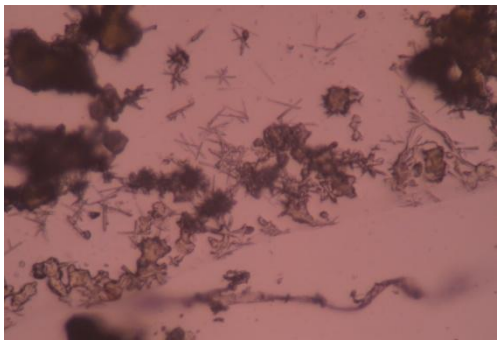

Figure no -5 Calcium Oxalte crystals

2.2.2 Physical test:

I .Color - Greenish yellow color was observed by visual inspection.[9]

II.Odour - Sweet odour was evaluated by smell of powder[9].

III.Loss on drying (LOD)-

It is an gravimetric method to determine the the moisture content in a powder.Weight of empty porcelain dish was taken and noted as W1-1.5g of powder was weighed in porcelain dish and weight was noted as W2.Porcelain dish containing 105 g powder was kept in an oven at 100 □ c until two consecutive readings of weight differ by more than 0.5 mg.[9]

There was no significant difference between three consecutive weight in gm taken at one hour interval. As per limit it should not be more than 0.5 mg ,values obtained are within the limits.

2.2.3 Chemical test:

I. Tests for Carbohydrates:

a) Molisch's test: The sample powder solution was treated with few drops of alcoholic α-napthol. Add 0.2 ml of concentrated H_2SO_4 slowly through the sides of the test tube, Purple to violet color ring appears at the junction.

b) Benedict's test: The sample powder solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) followed by boiling on water bath and reddish brown precipitate was observed to check reducing sugars are present or not.

c) Barfoed's test: (General test for monosaccharide) The test tube containing 1ml reagent and 1 ml of sample powder solution heated in a beaker of boiling water. Precipitate of red cuprous oxide was observed to check monosaccharides are present or not. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis into monosaccharide.[9]

II. Tests for steroids and triterpenoids:

a) Salkowski test: Sample was treated with 2 ml chloroform and 2 ml conc. H_2SO_4.After sometime, chloroform layer appeared red in the lower layer and acid layer shows greenish yellow fluorescence.[9]

III. Tests for reducing sugar:

a) Fehling's test: Mix 1 ml of Fehling's solution A and B and heat on a water bath for 2 minutes. Add sample powder. Heat on water bath for 5- 10 min. First yellow, then red ppt observed. [9]

IV. Test for anthraquinone glycosides:

a) Borntrager's test: Sample powder treated with dil.H_2SO_4, boiled and filtered. To cold filtrate, equal volume of benzene or chloroform was added. The organic solvent Separated and ammonia added. Ammonical layer turns pink or red.

b) Modified Borntrager's test:To sample powder add 5 ml 5% $FeCl_3$ and 5ml diluted HCl added, boiled and filtered. To cold filtrate, benzene or chloroform was added. Shake well. Organic solvent separated and equal volume of dilute ammonia added to it. Ammonical layer showed pinkish red color.[9]

V. Test for coumarin glycosides:

a) Powder when made alkaline, shows blue or green fluorescence

b) Take moistened dry powder in test tube. Cover test tube with filter paper soaked in dilute NaOH. Keep in water bath. After sometime expose filter paper to UV light it showed yellow-green fluorescence.[9]

VI. Test for alkaloids:

a) Mayer's test (Potassium mercuric iodide solution): 2-3 ml powder solution, add few drops of Mayer's reagent, creamy white precipitate is produced.

b) Dragendroff's test (Potassium bismuth iodide solution): 2-3 ml powder solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.

c) Wagner's test (Solution of Iodine in Potassium Iodide): 2-3 ml of powder sample solution; add few drops of Wagner's reagent, reddish brown precipitate is produced.

d) Hager's Test (Saturated solution of Picric acid): 2-3 ml of powder sample solution, add few drops of Hager's reagent, yellow precipitate is produced.[9]

VII. Tests for tannins compounds:

a) Lead acetate solution: Powder with lead acetate gives white precipitate.

b) Bromine water: decoloration of bromine water.

c) Dilute iodine solution : transient red colour.[9]

VII. Tests for phenolic compounds:

a)Ferric chloride test :Powder gives blue-green color with few drops of $Fecl_3$.

b)Dilute Potassium permanganate solution: decoloration.[9]

VIII. Tests for flavonoids:

a) Shinoda test (Magnesium Hydrochloride reduction test): Powder was treated with 5 ml of 95% ethanol, few fragments of magnesium turnings and concentrated hydrochloric acid drop wise so pink or red color appears after few minutes.

b) Sulfuric acid test : To the powder add water and few drops of Sulfuric acid; formation of an intense deep yellow solution .Chalcones and aurones gives red or red bluish solutions. Flavanes give orange to to red colours.[9]

Table no 1 Results of chemical tests.

Sr no.	TEST	OBSERVATION	RESULT
1.	Carbohydrates- a) Molisch's test	Voilet ring at junction	Carbohydrates present
	b) Benedict's test	Green color appears	Carbohydrates present.
	c) Barfoed's test	Red precipitate	Carbohydrates present.
2.	Steroids and Triterpenoids- Salkowski test	Acid layer shows green fluorescence	Steroids present
3.	Reducing sugar- Fehlings test	Red precipitate	Reducing sugar present
4.	Anthaquinone glycosides-	Ammonical layer do not show pink color	Anthraquinone glycosides absent

5.	Alkaloids- a)Mayer's reagent b)Dragendroff's reagent c)Wagner's reagent d)Hager's reagent	Precipitate not observed. Orange color do not observed. Reddish brown color not observed Yellow precipitate not observed	Alkaloids absent Alkaloids absent Alkaloids absent Alkaloids absent
6.	Tannins- a)Lead acetate solution b)Bromine water c)Dilute iodine solution	White precipitate not observed. No decoloration. No transient red color observed	Tannins absent Tannins absent Tannins absent
7.	Phenolic compound- a)Ferric chloride solution b)Dilute Potassium permagnete solution	No blue green color observed No decoloration observed	Phenolic compound absent Phenolic compound absent
8.	Flavonoids- a)Shinoda test b)Sulphuric acid test	Orange,pink,red,purple color not observed No orange color observed	Flavonoids absent Flavonoids absent

2.2.4 Pre-compression parameters:

I. Angle of repose(Ɵ) :

Funnel method: Funnel was attached to the burette stand at distance of two cm height from tip of funnel to graph paper placed on the surface. Sample powder was poured from upper side of funnel ,powder forms peak on graph paper. From the obtained diameter angle of repose (Ɵ) was calculated.

$$\theta = \tan^{-I}(h/r)\text{---------}(1)$$

Where h = height of funnel from tip to graph paper surface = 2 cm

r = radius of circle

Table no 2 Relationship between Angle of repose and flowability

Angle	Flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

II.Bulk density:(D_b)

$D_b = M/V_b$ ----------(2)

M = weight of powder (gm)

V_b = bulk volume (ml)

III.Tapped density :(Vt)

Tapped density was calculated using bulk density apparatus of model.Sample powder was poured into measuring cylinder and fifty tapping was given.Then the tapped volume was measured.

$D_t = M/V_t$ ----------(3)

M = weight of powder (gm)

V_t = tapped volume(ml)

VI. Carr's Index : (I)

Percentage compressability is calculated using following formula-

$I = D_t - D_b/D_t * 100$ ----------(4)

D_t = tapped density (ml)

D_b = Bulk volume (ml)

Table no 3 Relationship between % Compressibility and Flowability

% Compressability	Flowability
5-12	Excellent
12-16	Good
18-21	Fair passable
23-35	Poor
33-38	Very poor
More than 40	Very very poor

V. Hausner's ratio:

Hausner's ratio = D_t/D_b ----------(5)

D_t = tapped density (ml)

D_b = Bulk volume (ml)[6,8]

Table no 4 Result of Pre compression parameters

Sr no.	Parameter	Obtained value	Result
1.	Angle of repose	16.06□	Excellent flow
2.	Bulk density	0.49125 gm/ml	-
3.	Tapped density	0.60461gm/ml	-
4.	Carr's index	18.74	Fair passable
5.	Hausner's ratio	1.23	-

2.2.5 Analytical Evaluation:

I. pH Determination :

pH was determined using litmus paper. pH of dissolved powder sample in hot water was found to be slightly acidic in nature. [6,7]

II. Solubility:

10 mg of powder was taken and dissolved in 5 ml of various solvent like cold water hot water ,benzene ,di-methyl –sulphoxide , pet ether, ethanol Sonicated and observed visually to check solubility Powder sample was found to be swelled in hot water and slightly soluble in cold water and 0.1 N Hydrochloric acid.

[6,7]

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III. Infra-red (IR) spectroscopy:

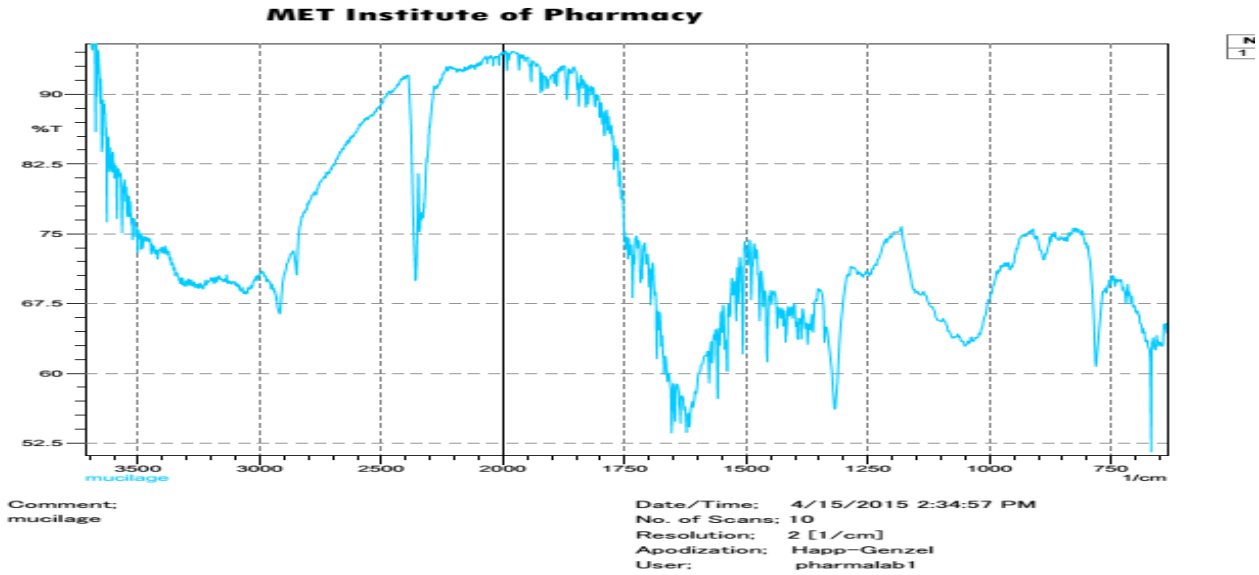

Figure no 6. IR spectra of isolated mucilage

Table no 5 Functional group with wave numbers

Reported wave number (cm^{-1})	Observed wave number(cm^{-1})	Functional group
1000-1500	107.29	C-O
1500-2000	1617.8	C-H stretch
3000-4000	3237.66	N-H stretch

VI. Fluoresence analysis : Powder sample was treated with various solvents like H_2SO_4,1N NaOH,methanol,water,HCL,$Fecl_3$ and observed under fluorescence chamber (make ,model) in day light, short UV light ,long UV light and color was observed.[10]

Table no 6 Results of Fluoresence analysis

Sr no.	TEST	Daylight	Short UV light	Long UV light
1.	Powder as such	Greenish yellow	Light green	Dark green
2.	Powder +H_2SO_4	Brown	Dark green	Black
3.	Powder +1N NaOH in Methanol	Brown	Light green	Brown
4.	Powder +1N NaOH in Water	Yellow	Yellow	Brown
5.	Powder +Methanol	Yellowish brown	Light green	Black
6.	Powder +$Fecl_3$	Brown	Brownish black	Black
7.	Powder +HCL	Green	Fluroscent green	Black

3.RESULT AND DISCUSSION

Mucilage obtained was evaluated using microscopic evaluation and organoleptic properties giving confirmatory test for presence of mucilage.Carbohydrates present in mucilage was studied using chemical test which is an essential group for glycosidic linkage in the mucilage. Solubility testing was carried out and mucilage was found to be swelled in hot water and insoluble in cold water hot water ,benzene ,di-methyl –sulphoxide, pet-ether, ethanol. The lk density and tapped density was found to be 0.49125 gm/ml and 0.60461gm/ml. By analytical investigation like IR spectra and fluorescence analysis mucilage was evaluated. [7,8]

4. CONCLUSION

It can be concluded that the isolated mucilage shows presence of Carbohydrates and reducing sugars which are responsible for the glycosidic linkage which are essential in sustaining the release effect. Hence isolated mucilage obtained from herbal plant can be used as pharmaceutical excipient. It may be used as gelling agent suspending agent or as an polymer in sustain release tablets. Mucilage can also be used as in treatment of ulcer.

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