Application of Seed Mucilage Extracted from *Lallemantia royleana* as a Suspending Agent

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Abstract

The mucilage from the seeds of *Lallemantia royleana* family Labiatae was extracted and subjected to preformulation study for evaluation of its suitability for use as suspending agent. Furosemide suspensions were prepared using (1.5% w/v) of the extracted *Lallemantia royleana* mucilage, (1.5% w/v) chitosan and (0.35% w/v) xanthan gum. The mucilage was white in color and the average yield of dried mucilage obtained from *L.royleana* nutlets was 14 % w/w of the seeds used. It is sparingly soluble in water but swells in contact with it, giving a highly viscous solution. It is slightly acidic to neutral. It was found that the extracted natural mucilage of *Lallemantia royleana* exhibited a higher viscosity profile and it exhibited better mucoadhesive property in comparison to chitosan, Carbopol 934 and hydroxypropyl - methylcellulose. The result showed that the suspension of furosemide prepared with 1.5 % w/v of the extracted mucilage was found to be ideal and comparable with the other two preparations of xanthan gum 0.35% w/v and chitosan 1.5% w/v. The study revealed that the mucilage of *Lallemantia royleana* has good properties to be used as a suspending agent and the performance is comparable with that of chitosan and xanthan gum since it is of natural origin, non-toxic, and of good biocompatibility.

Key words: mucilage; polymer; excipient; drug delivery; suspension; Lallemantia royleana.

الخلاصة

تستخدم الهلامات و الاصماغ النباتية الطبيعية بكثرة حديثا كسواغات في تصييغ الادوية. ان الغرض من هذه الدراسة هو استخلاص هلامة صمغية من بنيدقات (بذور) نبات البالنكو *Lallemantia royleana* ودراسة الخواص الفيزيائية و الكيميائية لهذا الهلام. اظهرت النتائج ان الهلام المستخلص هو مادة بيضاء و استخلصت بكمية ١٤% من الوزن الاصلي للبذور.كما ان تركيز محلول بتركيز ١% w/v من الهلام كان متعادلا الى قليل الحامضية. أشارت النتائج الى ان الهلام المستخلص ذو لزوجة عالية جدا و قابلية التصاق أعلى من الكيتوسان و Hydroxypropyl methylcellulose (HPMC) والكاربوبول carbopol حينما استخدموا بنفس التركيز (١% w/v). وأخيرا تم تحضير معلق الفيوروزيمايد بوجود الهلامة و بينت النتائج ان المعلق المحضر باستخدام تركيز (١.٥ % w/v) من الهلامة المستخلصة كان مقبولا و له خواص ثباتية و تحرر مشابهة او تفوق معلقين محضرين باستخدام الكيتوسان (١.٥ % w/v) و صمغ الزانثان xanthan gum (٠.٣٥% w/v). تبين الدراسة بالمجمل ان الهلام المستخلص من بذور نبتة البالنكو بالامكان ان يكون ملائما لتصييغ معلقات ذات خواص جيدة لتحرر الدواء كون هذا السواغ طبيعي , امن,متطابق, ذو خواص فيزيوكيميائية و صيدلانية جيدة .

Introduction

Natural gums and mucilage have been widely explored as pharmaceutical excipients such as thickeners, suspending agents, emulsifying agents, and binders [(1)]. It has been reported for the successful use of *Ocimum gratissimum*, *Butea monospermama* and *Leucaena leucocephala* seeds mucilage as suspending agent [(2).] *Lallemantia royleana*, commonly known as balango, is an annual herb belonging to the family Labiatae [(3)]. It is cultivated throughout Western Asia, India, Pakistan, and northern of Iraq, [(4)] *Lallemantia royleana* nutlets function as added palatable ingredient in cooling drinks and the highly mucilaginous nutlets have numerous applications in the traditional medicine; it is useful in abscesses as paste, inflammations and gastrointestinal problems [(5)] The nutlets are about 3 millimeter in length, 1 millimeter in breadth, dark-brown to black in color.[(6)] When moistened with water, they become coated with voluminous and translucent mucilage. The taste of the moistened nutlets is bland and somewhat spicy.[(7)] Many previous references disclose method of isolating various components from *Lallemantia royleana* nutlets, like volatile oils and mixed fatty acids. [(8)] The present study was undertaken with an objective to extract, evaluate and to find out the potentials as a suspending agent of natural mucilage obtained from the nutlets of the plant *Lallemantia royleana* (balango) .

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The application of plant mucilage as suspending agent explained by the gelling power and viscosity enhancing effects of these mucilages. For example , the formulation containing *Hibiscus cannabinus* mucilage as a suspending agent shows comparable results to that of standard marketed formulation .[9] Furosemide (loop diuretic) is practically insoluble drug; therefore, it was prepared as suspension using the extracted *L.royleana* mucilage as a suspending agent .Therapeutic agents of low water solubility could be suspended in a liquid suspending vehicle of the nutlets extract, which has an elevated viscosity, higher than that of water. [10]

Experimental
Materials and Methods

Furosemide from (Ajanta, India), Balango nutlets were purchased from local market in Baghdad, Iraq and the seeds mucilage was isolated in the laboratory as the balango seeds were soaked in water for 24 hours (in a ratio of 1: 20 w/v) . The mucilaginous seeds then blended in blender at low velocity for 10 seconds, and the mass was passed through muslin cloth. The mucilage was precipitated from the filtrate by adding 3 volumes of ethanol. The mucilage isolated from seeds was dried in an oven at 45°C for 2 hours. The powder samples were stored in tightly closed containers until used. [11] Chitosan from Fluka, biochemika , Switzerland. Xanthan gum from Merck, Darmstadt, Germany. All other materials used were of analytical grade.

Instruments

Instruments used are pH meter**(** Hanna,pH M-11microprocessor, Italy)**,** Spectrophotometer (Pu-1-pu pye unicam sp3-100 infrared, Unicam Ltd, Cambridge, UK), Dissolution apparatus(Paddle , Dis 6000, Copley Nottingham, UK), Viscometer (Brookfield DV -II cone and plate type .USA),

pH and viscosity measurements

The pH measurement was carried out for all mucilage dispersions. A dispersion of the mucilage (1% w/v) was used by taking 5 ml of the (1% w/v) gel and shaking it with 25 ml of water. The pH was estimated using Hanna pH Meter (model M-11). The mean of three determinations was calculated. The viscosity of 1% w/v solution of the extract was determined using Brookfield viscometer at different shearing rates at 30 ^{o}C. The same measurements for pH and viscosity were done separately for 1% w/v solutions of chitosan,hydroxypropyl methyl cellulose (HPMC), and carbopol for comparison.Further more, the viscosity of the extracted mucilage was tested at various pH ranges.

Characterization of mucoadhesive property of the mucilage

The mucoadhesive property of L.royleana mucilage as well as for chitosan was determined according to Park and Robinson method.[12] The Extracted mucilage was glued onto the lower platform of the equipment for mucoadhesive determination. An excised sheep duodenum measuring 2 cm width by 3 cm length was attached to the arm of the equipment by means of glue. The mucosa was gently brought into contact with the moistened disc and adhesion was allowed to take place for 5, 10, 15, 20, and 25 minutes. At the end of time intervals, the mucosa was gently detached from the mucilage disc and the force was directly recorded depending on the weight recording as detachment occurred on an electronic balance. The mean of three determinations was obtained.

Equilibrium swelling study

To 1 gm of the dried mucilage, 25 ml of water was added in a 30 ml graduated cylinder and the mixture was shaked thoroughly every 10 minutes for 2 hours, allowed to stand for 24 hours at room temperature. Then the volume in ml occupied by the mucilage was measured. The mean value of three determinations was recorded. [13] The equilibrium swelling studies were carried out for the gels at 37°C in buffer solutions of pH 2.1 and 7.4 (simulated gastric and intestinal fluids pH, respectively).

Retaining properties when heated

To study whether the extract maintains its characteristics when heated and cooled, a (1% w/v) viscous solution of *L.royleana* mucilage was subjected in to two cycles of heat and cool, at 100 C^{0}. [14]

Preparation of furosemide suspension

Three formulas of (2.5% w/v Furosemide) suspensions were prepared using the regularly used concentrations of appropriate viscosities, the extracted *L.royleana* mucilage (1.5 % w/v), chitosan (1.5 % w/v) and xanthan gum (0.35 % w/v) as suspending agents.

Dissolution of (2.5% w/v) furosemide suspension using L.royleana mucilage as a suspending agent

The United States pharmacopoea (USP) rotating – paddle dissolution apparatus (Copley) was used to study drug release from the furosemide suspension.Five ml of the

suspension equivalent to 125 mg of furosemide measured and suspended in 900 ml of phosphate buffer pH 6.8,was stirred at 50 rpm and 37 ^{0}C. At specific time intervals, samples (5 ml) were withdrawn and filtered. The same volume (5 ml) of the phosphate buffer pH 6.8 was replaced after each sampling. The drug content in the filtrate was determined by spectrophotometer at its λmax (271 nm).[15]

Sedimentation Parameters

The sedimentation volume were determined by keeping 50 ml of each suspensions in stoppered measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at time intervals of 1 day up to 35 days. [16]

Redispersion

Fixed volume of each suspension (50 ml) was kept in calibrated tubes which were stored at room temperature for various time intervals of 5days; one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any was recorded.[17]

Results and Discussion

The mucilage extracted from *Lallemantia royleana* seeds was found to be swells in contact with water, giving a highly viscous solution. It is slightly acidic to neutral. It was found that the extracted natural mucilage of *L.royleana* exhibited a higher viscosity profile than other tested polymer (505 cps [natural mucilage of *L.royleana*], vs. 187 cps [chitosan] vs. 65 cps [carbopol 934], vs. 20 cps [HPMC] respectively) at a concentration equivalent to (1 % w/v) as shown in figure (1). It appears that the extracted mucilage exhibited thixotropic (shear-thinning) behavior,figure(2). A marked dependence of the viscosity on pH was observed ,figure (3).i.e. As the pH increases the viscosity increases ($p < 0.05$) . Similar results were obtained for the mucilage extracted from the pods of *Abelmoschus Esculentus* when it was used as a suspending agent in Paracetamol Suspension. [18] Swelling indices of *L.royleana* mucilage powder (1 gm) at pH 2.1 and pH 7.4 were found to be 7 and 25, respectively. The data indicated that the swelling of mucilage is pH-dependent, and the mucilage is anionic and this property is of value since the excipient support the drug to be retained to the intestine , the site of maximum absorption of the active constituent. [19] Furthermore, the result showed that the extracted mucilage exhibited higher adhesion and better mucoadhesive property in comparison to chitosan, Carbopol 934 and (HPMC) as shown in figure (4).The rheological behavior of the suspensions prepared with mucilage of *Lallemantia royleana*, chitosan and xanthan gum are given in figure. (5). The results reveal that the suspensions are pseudoplastic in their behavior and their viscosity decreases with increase in shear rate, which is an essential requirement in the formulation of suspension. [20]

Suspending properties of L.royleana mucilage

The extracted mucilage was found to be comparable to chitosan and xanthan gum as suspending agent. The results obtained indicated that the extracted mucilage may be used as a source for pharmaceutical adjuvant specifically as a suspending agent. It has been observed that 100% drug was released within 15 minutes in case of the suspension using the extracted *L.royleana* mucilage (1.5 % w/v). The same results was obtained for suspension containing xanthan gum (0.35%w/v), and slightly faster than that contains chitosan (1.5 %w/v) as a suspending agent (released within 20 minutes) as shown in figure (6), table (1). Statistical analysis was performed; one-way analysis of variance (ANOVA) of percentage released among the three groups was studied. The linear regression analysis performed on the square root data is shown in the figure (7). The regression lines produced by both the extracted *L.royleana* mucilage and xanthan gum suspensions are almost identical while the line produced by chitosan suspension is statistically different ($p < 0.05$).Sedimentation was followed over a period of 25 days and almost no sedimentation was seen. The exhibition of excellent suspending properties of the extracted L.royleana mucilage are not completely dependent on viscosity, an example of suspensions prepared using some higher viscosity hydrocolloids, carboxymethylcellulose, settle out of solution on standing.[21] Similar studies on the suspending power of other plant mucilage were reported by Mital and his co-workers and they concluded that 1% *Albizia zygia* mucilage have the same suspending power as 0.4 % tragacanth. [22] In conclusion *Lallemantia royleana* mucilage was found to have acceptable physicochemical and drug release properties; it is of natural origin, non-toxic, biocompatible and cheap. Therefore, it is suitable for formulation of suspension preparations. Also the suspension prepared with 1.5 % w/v of the extracted *L.royleana* mucilage was found to be ideal and comparable with two preparations of xanthan gum 0.35% w/v and chitosan 1.5% w/v suspensions.

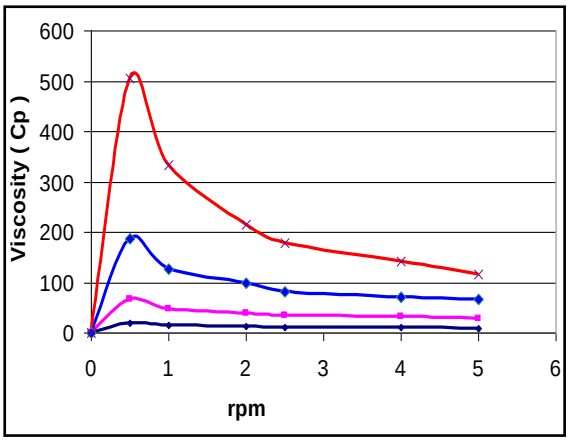

Figure 1 : Comparative evaluation of viscosity of the extracted L.royleana mucilage (—×—), chitosan (—■—), the synthetic polymers carbopol 934 (—▪—) and HPMC (—▪—) .Using 1% w/v solution at 30°C±1. Values are expressed as the mean of 6 observations. speed of viscometer ; rpm(round per minute)

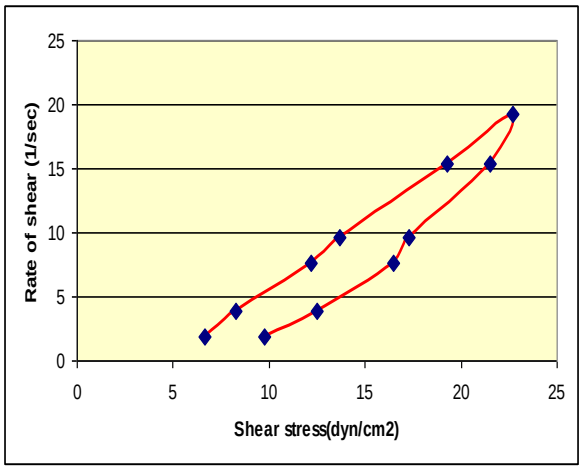

Figure 2: Plot of rate of shear vs. shear stress for 1 %(w/v) solution of the extracted L.royleana mucilage.

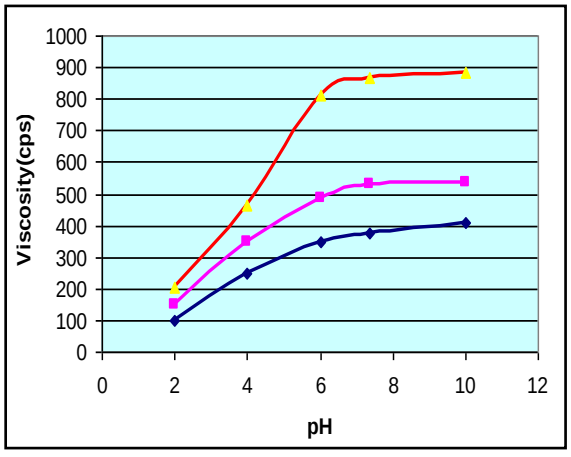

Figure 3 : Effect of pH on viscosity of various concentrations of the extracted L.royleana mucilage.(—♦—) 0.5% ,(—■—) 1%, (—▲—) 3% w/v .

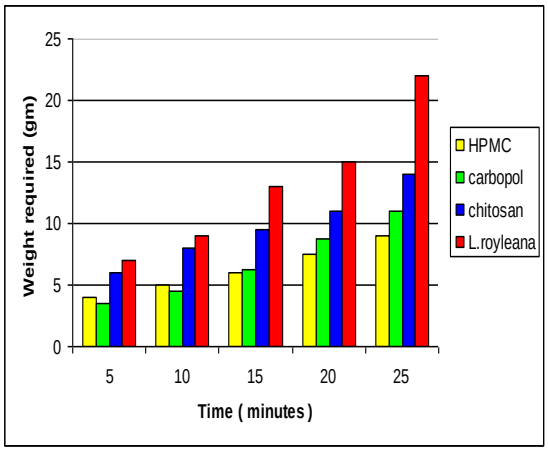

Figure 4: Mucoadhesion of (1% w/v) gel , of the extracted L.royleana mucilage■ ,chitosan ■, synthetic polymers carbopol 934 ■ and HPMC■.

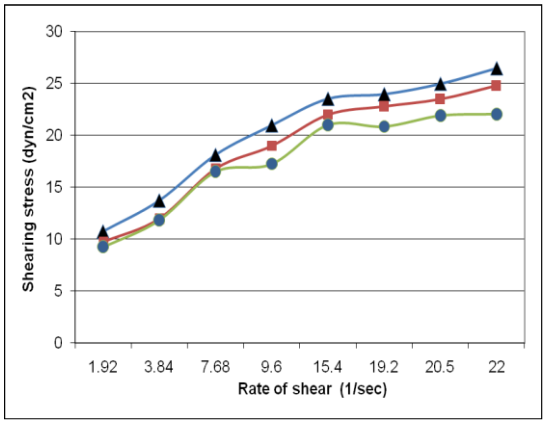

Figure 5 : Rheological behavior of the suspensions , of the extracted L.royleana mucilage ,1.5 % (w/v) ▲ ,chitosan 1.5 % (w/v) ■, and xanthan gum 0.35% ●.

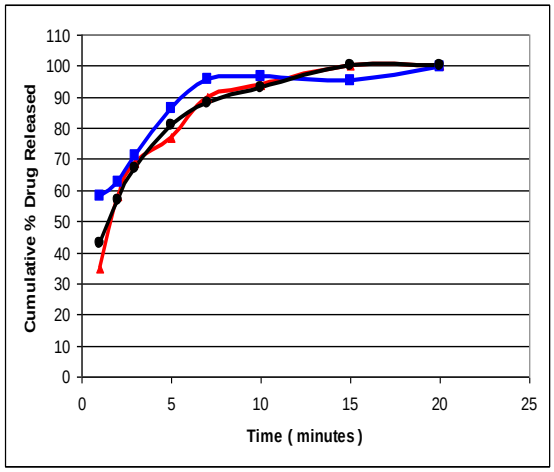

Figure 6: Release profiles of furosemide (2.5 % w/v) from suspensions L.royleana —▲— (1.5% w/v), chitosan —■— (1.5% w/v) and xanthan gum —●— (0.35%) .At pH 6.8,and 37°C.

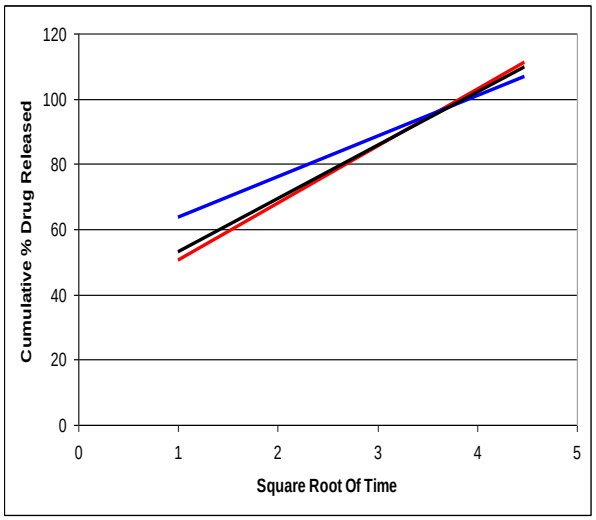

Figure 7 : Higuchi plots of furosemide **release profiles from suspensions; L.royleana — (R^2 0.8458), chitosan — (R^2 0.8176) and xanthan gum — (R^2 0.885).**

Table 1: Dissolution of 2.5% w/v Furosemide Suspension in Different Suspending Agents.

Time (minutes)	Percent furosemide released ,the suspending agent is 0.35% w/v xanthan gum	Percent furosemide released, the suspending agent is 1.5 % w/v chitosan	Percent furosemide released ,the suspending agent is 1.5 % w/v L. royleana mucilage
1	43	58.2	35
2	57	62.5	58
3	67	71.1	69
5	81	86.3	77
7	88	95.6	90
10	93	96.4	94
15	100	95.3	100
20	100	99.8	100

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