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Single-step Method for the Extraction of Mucilage from *Ocimum americanum L.* Seeds

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Abstract: In this work, a single-step extraction method for removing the mucilage present in *Ocimum americanum L.* seeds was studied. The effect of various parameters on the yield of mucilage such as seed water ratio, speed of the agitator and temperature was also studied. Initially all the experiments were conducted in a 0.5-L glass beaker, and optimum parameters were determined. It was observed that temperature, speed and seed water ratio play an important role in the yield of mucilage. Then the experiments were extended in a 1.5-L stainless steel vessel designed for the above purpose. The kinetics of mucilage extraction at optimum conditions were also studied. It was found that there was a linear relationship exists between the yield of mucilage and time. The study further reveals that at optimum conditions almost complete recovery of mucilage will be possible within 50 minutes of extraction process.

Keywords: *Ocimum americanum* L. seeds, Mucilage, Extraction.

Introduction

Ocimum americanum L. is an important herbal plant which is growing in many places of India. The seeds of this plant contain a thin outer layer having a larger capacity of hydration. The seed grows within seed pods each of which contains 4–8 small seeds. In the maturation of seed pods, the seeds grow within a jelly-like substance. As the seeds mature, the mucilage dries onto the outer hull of seeds as a thin layer. When the seeds are choked in water, outer layer of seeds swells by absorbing the water. The swollen outer layer appears as a white gel called mucilage.

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O. americanum L. (*Syn. O. canum*), hoary basil or mosquito plant has three distinct chemo-types (floral-lemony, camphoraceous and spicy). The whole part of the plant such as leaves, seeds and seed pods was having various applications. The essential oil obtained from the leaves of this plant ~~was~~ active against various microorganisms [1]. It was also reported that the essential oil obtained from the plant can be used for pest control applications [2].

The hydrocolloid or mucilage from *O. americanum* L. "Lemon" was solely composed of carbohydrates, with Alkali-soluble hemi cellulosic polysaccharides (81.9%, w/w) containing 18.1% uronic acid and cellulose (27.6%, w/w), indicating that the fibrous material was a physical composite of cellulose and hemi-cellulosic polysaccharides. The degree of polymerisation of cellulose was determined to be around 3,000 as a nitrate. Weight average molecular mass of the alkali-soluble material was estimated to be 68.0 kDa [3].

The mucilage acts in a similar manner to polysaccharide gums such as glucomannans and pectin. Mucilages are most commonly used as an adjuvant in pharmaceutical preparations, with a wide range of applications such as thickening, binding, disintegrating, suspending, emulsifying, stabilising and gelling agents [4]. The ~~small amount of dried~~ mucilage is very effective ~~when~~ increasing the viscosity of solutions at levels as low as 0.5–1% [5]. It was reported that the *O. americanum* L. mucilage have good disintegrant properties than the starch [6].

In the literature, several reports are available on the extraction of mucilage from the mucilaginous seeds [7–9]. But the disadvantage of those methods is lower yield of mucilage after the extraction process. And there was a difficulty in separating the mucilage from aqueous solution by those methods. While the literature describes the nature of the *O. americanum* L. mucilage well, they lack in explaining the complete extraction of mucilage from the seeds in a simple-step extraction method. All previously published methods are economically unsuitable for industrial scale. A rapid and effective method is required for extraction of mucilage from *O. americanum* L. seeds, but the factors affecting the extraction of this type of mucilage were not known. This work was aimed at filling this void in extraction of mucilage from *O. americanum* L. seeds.

Experimental

Materials

Source of O. americanum *L. Seeds*

The *O. americanum* plant seeds are harvested from the village Pettavaithalai, Trichy and Keelapoolanandapuram, Theni district. The plants are widely available in all places of Tamil Nadu. They are having a good drought-resistance capability. The seeds are collected from the plants, and then seeds are separated manually by skilled labours.

Agitator Set Up and Other Accessories

Glass beaker (500 ml) and 1.5 stainless steel vessel, speed regulator, motor (max speed: 4,000 rpm, ~~0.5~~ HP), Tachometer to measure speed, ~~demonised~~ water from Milli-Q unit, heating plate with temperature settings, mercury glass thermo meter, stainless steel sieves.

Method of Mucilage Extraction

The mucilage can be separated by agitating the seeds–water solution in a vessel. The speed of agitator, temperature, seed–water weight ratio and addition of chemicals are the factors which will influence the yield of mucilage. The friction between seed-wall of agitator vessel, seed–seed, seed–impeller will release the swollen white gel from the seed and diffused into the water phase.

Calibration of Speed Regulator

The agitator speed was measured after reaching the steady state by using the Tachometer. The regulator position was adjusted to the speeds 200 rpm, 500 rpm, 800 rpm, 1,000 rpm, 2,000 rpm, 3,000 rpm and 4,000 rpm by using water as a medium, and markings were done on the regulator. All the experiments were conducted at the speeds vary between 200 rpm and 3,000 rpm.

Determination Yield of Mucilage

About W_2 grams of dry seeds was taken in a 500-ml glass beaker, the required volume of water was added and the seeds were allowed to swell for the required time. The speed of the agitator was set at 3,000 rpm. The stirring was continued for 30 minutes. The mucilage–seed solution was passed through the muslin cloth and separated by ordinary separation. The mucilage solution was poured into the tray made from aluminum foil. The solution was continuously dried in a hot air oven set at 45°C. Then the dried mucilage was cooled in a desiccator to room temperature and immediately weighed. The yield of mucilage after extraction of mucilage was calculated by using the following formula:

$$\text{Yield} = \frac{W_1}{W_2}$$

where W_1 – gram of dried mucilage and W_2 – gram of dry seed taken.

Results and Discussion

Effect of Temperature on Mucilage Yield

To study the effect of temperature, two experiments were conducted. One is at room temperature and another one is at 60°C. The temperature was also an important parameter in the extraction of mucilage. The yield of mucilage at 30°C was 13.8%. But when temperature was increased to 60°C, the yield of mucilage increased to 23.16%. From Table 2, it is clear

Table 1. Effect of temperature on mucilage yield

Volume of water (ml)	Time of agitation, (min)	Temperature (°C)	Speed of agitator (rpm)	Weight of dry seeds W_2 (gm)	Yield of mucilage W_1 (gm)	Yield (%) $\frac{W_1}{W_2} \times 100$
300	45	30	3,000	5	0.69	13.8
300	30	60	3,000	5	1.158	23.16

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Table 2. Effect of seed–water ratio on mucilage yield

Weight of seeds (X), g	Volume of water taken (ml)	Volume of filtrate solution collected (ml)	Dry mucilage weight for 30 ml of mucilage water solution (gm)	Total dry mucilage weight, Y, (gm)			Average mucilage weight (gm)	% Extraction = 100 × Y/X
				1	2	3		
1	300	280	~~0.025~~	0.21	0.2	0.2	0.2	20
3	300	270	0.074	0.66	0.67	0.67	0.66	22
5	300	160	0.131	0.69	0.7	0.68	0.69	13.8
7	300	80	0.068	0.18	0.19	0.19	0.19	2.7
10	300	0	–	–	–	–	–	–

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that the time required for the extraction of mucilage is also decreased considerably when extracting the mucilage at 60°C.

Effect of Seed–water Ratio on Yield of Mucilage

Required quantity of seeds (1, 3, 5, 7 and 10 g) was taken in a 500-ml glass beaker. Then 300 ml of water was added. Seed–water solution was agitated at the speed 3,000 rpm for 15 minutes. The seed–water solution was filtered through a stainless steel mesh filter to get mucilage. The mucilage was dried to determine weight of mucilage. The extraction of mucilage first increases with seed–water ratio. But it decreased towards zero when the water–seed ratio was 0.033. The reason for this is that the higher the water content, the lower the diffusion resistance of mucilage from seed to solution. During the extraction, the mucilage will move from seed to water. When the seed–water ratio is high, all the water will be absorbed by the seed outer layer. The viscosity of the solution is too high. Also the volume of free water will decrease and this makes the diffusion limitation during the extraction. The optimum seed water ratio was found to be 0.0133 (4 g seed/300 ml water).

Effect of Speed on Mucilage Yield

Four grams (optimised in previous experiment) of seeds was taken in a vessel and then 300 ml of water was added. Seed–water solution was agitated at different speed ranges from 500 to 3,000 rpm about one hr. Then the seed–water solution was filtered through a stainless steel mesh filter to determine the weight of mucilage.

The speed of the agitator increases the centrifugal friction between the vessel wall–seeds, impeller–seeds and seeds–seeds. So the extraction efficiency was increased. The yield of mucilage increases approximately linearly up to the agitator speed 2,000 rpm. But after that there was no drastic increase in the yield. This was the expected phenomena. At higher speed, the fluid behaviour will be turbulent mixing. Above 2,000 rpm, power absorbed by the liquid phase will be approximately the same, and there was no more increase in shear force. Hence the yield of mucilage will move towards the constant value after 2,000 rpm. From Table 3 it was concluded that the optimum speed to get the maximum shear force was 2,000 rpm. Since the viscosity of the solution varies during the course of extraction process, the actual speed measured by the Tachometer is less than the speed value marked in the speed regulator apparatus. The speed values given in Table 3 were measured by Tachometer during the extraction process. Definitely when the speed of agitator increases, the temperature of the

Table 3. Effect of agitator speed on mucilage yield

Seed weight (gm)	Speed (rpm)	Volume of water (ml)	Time (min)	Volume of filtrate solution (ml)	Dry mucilage weight (gm)
4	500	300	60	130	0.019
4	1,000	300	60	180	0.0539
4	1,500	300	60	195	0.0579
4	2,000	300	60	240	0.064
4	3,000	300	60	265	0.069

aqueous medium also increases. But the increase in temperature will be less than 5°C. And it was also observed that this change of temperature of liquid phase will have only little effect on increasing the yield of mucilage.

Kinetics of Mucilage Extraction

After understanding the effect of temperature, seed–water ratio, speed of the agitator in a 0.5-L glass beaker, it was decided to conduct the experiments in a 1.5-L stainless steel vessel. About 15 g of seeds was taken in a 1.5-L stainless steel vessel. Then 1,000 ml of water was added. Seed–water solution was agitated at 2,000 rpm. The yield of mucilage was estimated by taking 100-ml well mixed seed–water solution from the vessel. The 100 ml of seed–water solution was filtered through a stainless steel mesh filter. The volume of filtrate was determined per 100-ml solution. About 30 ml of clear filtrate was taken and poured into a pre-weighed aluminum tray. This was kept in a hot air oven at 45°C to determine the mucilage dry weight per 100 ml of the solution. The remaining filtrate and seeds on the stainless steel mesh were again put into the 1.5-L mixing vessel. To compensate the 30 ml of clear mucilage taken out from the vessel, about 30 ml water was added into 1.5-L mixing vessel. The same steps were repeated for every 5-minute interval.

The yield of mucilage at different time intervals was studied and tabulated in Table 4. Almost all the mucilage has been extracted within 50 minutes. It was very clear from the graph shown above after 40 minutes; there was no change in the yield of mucilage. The yield of mucilage is comparatively higher (23–26%) than the mucilage yield (14%) which was reported earlier [6]. It was observed that there was a sharp increase in the yield, when the time was less than 5 minutes. It was also noted that there was a liner relationship exists between the yield of mucilage and the time of extraction.

From the table it was also observed that there was a sharp increase in the yield of mucilage for the first 30 minutes of extraction. After that the extraction rate slowly decreases and reaches the constant value. The volume of filtrate is increased gradually from 350 ml to

Table 4. Kinetics of mucilage extraction

Time (min)	Volume of filtrate (ml)	Dry mucilage weight (gm)
5	350	1.05
10	360	1.37
15	400	1.91
20	470	1.86
25	500	2.34
35	550	2.72
45	600	3.11
60	640	3.37
70	670	3.74
80	730	3.97
90	750	3.9
100	770	3.8

770 ml when time increases. It can be observed from Table 4 that after 50 minutes of extraction, there is no more increase in the concentration of mucilage. After 50 minutes, the concentration of mucilage attached with seed and the concentration of mucilage in filtrate solution were nearly the same. Hence, continuing the extraction after 50 minutes will not further increase the yield of mucilage per unit volume. The shear force between the seeds–wall of the agitator vessel is a main factor in determining the yield of mucilage. The wall of the agitator vessel used in this experiment was smooth. If the wall surface of the vessel is roughened by some method, it will increase the shear rate between the seeds and the wall surface of the vessel. This will result a considerable reduction in the time of extraction of mucilage from seeds.

Conclusion

The mucilage from the seeds was separated by using agitator set-up. The optimum seed–water ratio was found to be 0.0133. The temperature plays a key role in separation. It considerably reduces the time of extraction and increases the yield of mucilage. The optimum speed of the agitator was 2,000 rpm. The kinetic study reveals that at optimum conditions almost complete recovery of mucilage will be possible within 50 minutes of extraction process. In this paper, only the parameters affecting the extraction were discussed. To improve the extraction, roughening the wall surface of agitator vessel is a good idea and is expected that this modification results in a further reduction of time in mucilage extraction process. Since this is a preliminary study, further optimisation studies are required to validate the above statement.

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