Original Article

Extraction and Evaluation of Lipid Entrapment Ability of *Ocimum basilicum* L. Seed Mucilage

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ABSTRACT

Background: Overweight, obesity is a serious health problem worldwide and has been becoming an epidemic recently due to the rapid growth of its prevalence. The consequences of obesity are not only its related diseases such as diabetes and cardio vascular disease, but also the effects on global socio-economic. **Aim:** In this study, we obtained and evaluated characteristics of *Ocimum basilicum* L. (OB) seed mucilage which fit for developing a weight control product through several characteristics such as swelling index, stability, toxicity and lipid adsorption/absorption from different sources. All of the statistical analysis was done by Graphpad prism using unpaired *t*-test. **Results:** The extraction yield by physical method was 10.17% and the swelling index of dried mucilage was 20.6 times higher than the initial weight. The drying temperature affected only on the mucilage through the increasing of reducing sugars concentration. The OB mucilage showed neither cytotoxicity on NIH-3T3 cells nor chronic/acute toxicity on mice. Last but not least, OB mucilage was able to absorb/adsorb lipid from different lipid sources. **Conclusion:** OB mucilage was proven to be a high potential source of polysaccharide for developing weight treatment products.

Key word: High swelling index, Lipid adsorption/absorption, Mucilage characteristics, *Ocimum basilicum* L., Obesity.

INTRODUCTION

Overweight, obesity has been becoming the major health issue all over the world, when approximately 39% of adults were overweight and 13% of them were obese in 2016 (WHO annual report). The increasing in prevalence of overweight and obesity in both developed and developing country, from 1980-2013, put a warning to the world health problem.[1,2] With this continuous trend all over the world, nearly 60% of the world population will be considered as overweight and 20% among them will be obese.[3] Along with type 2 diabetes, obesity resulted in several diseases such as hypertension, hypercholesterolemia, type 2 diabetes mellitus, CHD and stroke.[4] Because of these obesities linked diseases, the estimated healthcare cost for the overweight (BMI > 27.5) went up 20% compared to normal people.[5] Moreover, an addition of $16 billion would be spent during the years 1996-2021 on healthcare cost by overweight and obese women.[6]

Overweight, obesity is a complicated issue, which is the consequence of our modern lifestyle, particularly the closing of energy balance.[7-10] Nonetheless, the booming of the fast food industry has made our problem even worse.[11,12] With such a global burden, establishing novel products for weight control, especially obesity control would best fit for the situation.

Seeds mucilage, such as Flax seeds (*Linum usitatissimum*), Chia seeds (*Salvia aegyptiaca* family), *Mimosa pudica* (*Mimosaceae*), etc, has been widely used since the ancient time, mostly in drugs and in cuisine. Among that, sweet basil (*Ocimum basi-*

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licum L.) is a common plant in Asian countries, whose leaves has been used daily as spice herb in culinary.[13] Besides that, sweet basils are also applied in medical treatment for headaches, coughs, diarrhea, constipation, etc.[14] More important, the ability to control weight of basil seeds was recorded in Pharmacopoeia, but there still no studies conducted on this. The seed mucilage, whose majority component is polysaccharide, possesses several characteristics that could be studied and applied to a vast area, including functional food for preventing obesity. Firstly, the polysaccharide has the bulking ability, which can occupy the stomach space and thus prevents the hungers. Second, the polysaccharide consists of many sugar chains, which can form a hollow structure to entrap different molecules. And last, polysaccharides that can't be digested in human gut are a good fermented source for beneficial bacterial; therefore they might have effects on the energy intake mechanisms.

In this study, we extracted and examined some characteristics of sweet basil mucilage, for further development of a novel obesity control product.

MATERIALS AND METHODS

This study, from January 2019 to July 2019, was conducted in the Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh city, Vietnam. Basil (*Ocimum basilicum* L.) seeds supply was from Khanh Hoa province, Vietnam.

Mucilage extraction from basil seeds

Mucilage was obtained from seeds of *Ocimum basilicum* L. using the methods described by Razavi[15] and Meghana[16] with some modifications. A Basil seed (approximately 10 g) was soaked in 300 mL of distilled water (at 67°C in 20 min). The mixture was stirred in order to separate the mucilage from seeds and later passed through a filter cloth to retrieve the mucilage. The separated mucilage was dried at 45°C in approximate 2 days to obtain dried mucilage. The yield was determined by the ratio of dried mucilage/seeds input mass.

Determining the swelling ratio and stability of dried mucilage in different pH solutions

Dried mucilage (10 mg) was soaked in 1 mL of distilled water (in 1.5 mL centrifuge tubes) and let still overnight to obtain the maximum swelling ratio of mucilage. The spare water was removed via centrifugation at 3000 rpm in 5 min and pipetting. Afterwards, the swollen weight was determined and swelling ratio was calculated following this equation:

$$\text{Swelling ratio} = \frac{\text{weight of swollen mucilage}}{\text{weight of dried mucilage}} (\text{times}) \quad (1)$$

The stability of mucilage was determined using the same method, but with slightly modification. In particular, dried mucilage was soaked in pH-adjusted distilled water (at pH of 1.5, 4.5 and 6.5).

Evaluation of reducing sugar concentration of dried mucilage using Dinitro Salicylic Acid (DNS) method

Reducing sugars were able to reduce 3,5-dinitrosalicylic acid (DNS) in alkaline solution into 3 amino 5 nitro salicylic acid. The reducing sugars concentration contained in dried mucilage was quantified following the protocol described by Saqib and Whitney.[17] In brief, dried mucilage was homogenized in water at 10 mg/mL concentration IKA Ultra Turrax T8 Homogenizer. The solution was then incubated with DNS reagent (1:1 volume ratio, total of 6 mL solution in a glass tube) in 20 min at 95°C. The absorbance at 540 nm was measured.

In vitro cytotoxicity of dried mucilage on NIH-3T3 cell line

In vitro cytotoxicity of OB was evaluated using the NIH-3T3 cell line and MTT tetrazolium. NIH-3T3 cells were cultured in DMEM-10 medium (Himedia, USA). Cells were then transferred to 96-well plate at a density of 110^4 cell/mL in 100 µl DMEM-10/well, incubated in 24-hour at 37°C, 5% CO_2. Then the medium was removed and replaced with 100 µl DMEM-10 containing OB mucilage at different concentrations (0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 mg/mL), incubated at 37°C, 5% CO_2 for 48 hr. MTT tetrazolium reagent was then added at the ratio of 1/10 (v/v) and incubated for 3 hr, allowing live cells to convert tetrazolium to formazan. The medium was then removed and the formazan precipitate was dissolved with 100µl DMSO and measured by absorbance at 550nm with Multiskan Ascent. The experiments were performed with 3 biological replicates.

The percent of live cell was calculated using the following formula:

$$\text{Live cell ratio} = \frac{\text{OD (well added mucilage) was}}{\text{OD (well no added mucilage)}}$$

Log IC_{50} (mg/mL) = x, which was determined by equation y = -43.339x + 64.407. (2)

Log LD_{50} (mg/kg) = 0.372 log IC_{50} (µg/mL) + 2.024.[18] (3)

In vivo acute and chronic toxicity on Swiss mice

Acute toxicity was evaluated on mice (n=3) at an OB dose of 250 mg/kg/day and followed up within 48 hr after oral administration. For evaluation of chronic toxicity, six 4-week old mice with weight of about 20-25 g were randomly divided into two experimental groups (n=3). Test group received the OB dose of 250 mg/kg/day and the control group received Phosphate Buffered Saline (PBS) as placebo. The experiment lasted 30 days. Mouse daily behaviors were monitored. Body weight was measured every week. After 30 days of oral administration, blood was collected to test for hematological factors which were determined by automatic biochemical analyzer (BioSystems, Spain).

Lipid adsorption/absorption capability of mucilage from Ocimum basilicum using sulfo-phospho-vanillin colorimetric method

Dried mucilage (10 mg) was soaking and incubated with either coconut oil or butter solution (weighted 100 mg and dissolved in 1 mL of distilled water) in a centrifuge tube 1.5 mL, at 37°C in 30 min in a shaking incubator. Coconut oil and butter without incubation with OB was used for control. After incubation, 100 µL of the excess oil (the layer above) was retrieve to perform quantification using vanillin reagent described by Cheng *et al*.[19] The amount of lipid adsorbed/absorbed was determined by comparing differences between concentration of samples (with or without mucilage). The oil to be evaluated (100 µL) was mixed with 100 µL of solvent (chloroform: methanol with volume ratio of 2:1) and 100 µL of the mixture was transferred to a 96-wells plate. Samples were incubated at 90°C in 2 min for solvent to evaporate. Then, 100 µL of acid sulfuric (98%) was added and continued to incubate at 90°C in 20 min. Reaction temperature was quickly cooled down to room temperature in 2 min and OD 540 nm was measure for blank condition (before reagent was added). 50 µL of phosphor vanillin reagent (0.25 mg/mL of vanillin in 68% phosphoric acid) was added and samples were incubated for 10 min for the reaction to take place. Finally, OD 540 nm as measured.

Statistical analysis

All of the Statistical analysis was done with GraphPad Prism version 7.0 software (GraphPad software Inc., La Jolla, CA, USA).

RESULTS

Mucilage extraction from Basil seeds (Ocimum basilicum L.*)*

The extraction yield of basil seeds mucilage using physical forces (stirred and filtered) was approximately 10.17 ± 0.36% (Table 1). Dried mucilage had several characteristics shown in Table 2. There was a change in the appearance of dried mucilage compared to the original. In particular, mucilage after passed through filter cloth had a white color and non-transparent. The color of dried mucilage changed to chamois color (Supplement Figure 1).

Table 1: Basil seed mucilage extraction yield.

	Replicate		
	1st	2nd	3rd
Basil seed mass (g)	10.32	10.15	10.11
Mucilage mass (g)	1.03	1.01	1.07
Yield (%)	9.98	9.95	10.58
Mean ± SD	10.17 ± 0.36		

Table 2: Characteristics of mucilage before and after dried.

Characteristics	Before	After dried
Odor	Non	Non
Color	White	Chamois
pH	6.80 – 7.20	6.80 – 7.00

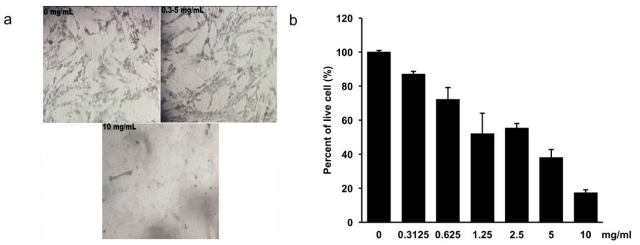


Figure 1: The toxicity of OB are evaluated *in vitro* and *in vivo*. (a) NIH-3T3 cell line in different dose of OB. The ratio of survived NIH-3T3 cell in different dose of OB; (b) Percent of live cells measured at different dried mucilage concentration.

The swelling ratio of dried mucilage

The swelling ratio of dried OB mucilage (w/w) was shown in Table 3. According to the result, dried mucilage could absorb the amount of water that was about 20 times higher than its' initial weight, which was a high swelling index.

Besides that, when soaked in different pH solutions (pH = 1.5; 4.0 and 6.5), OB mucilage still showed the same swelling index as in distilled water alone (Table 4), proven the stability of OB mucilage in digest system stimulated pH solutions.

Table 3: The swelling ratio of OB mucilage.

	1st	2nd	3rd	4th	5th
Initial weight (mg)	10	10	10	10	10
Swollen weight (mg)	200	190	220	200	220
Swelling index (times)	20	19	22	20	22
Mean ± SD	20.60 ± 1.34				

Table 4: The stability of OB mucilage in different pH solutions.

pH	Swelling index (times) (3 replicates)			Mean ± SD
1.5	23.76	14.41	14.47	17.55 ± 5.38
4.0	27.13	24.99	26.55	26.22 ± 1.11
6.5	24.05	19.89	30.59	24.84 ± 5.39

Table 5: Reducing sugar concentration of raw and dried mucilage.

Sample (mg/mL)	10		
Raw mucilage (µg/mL)	20.31		
Dried mucilage (µg/mL)	751.03	740.63	726.03
Mean ± SD	739.23 ± 12.56		

Reducing sugar concentration of dried mucilage

In order to quantify the reducing sugar concentration in dried mucilage, we generated a standard curve based on glucose and DNS reagent. The concentration of mucilage samples before and after dried was shown in Table 5. The concentration of reducing sugar in dried mucilage was 739.23 ± 12.56 (µg/mL), which was nearly 37 times higher than the original with 20.31 (µg/mL).

In vitro cytotoxicity of dried mucilage on NIH-3T3 cell line

In order to identify the safe dose of OB, we first tested it with *in vitro* toxicity assays using NIH-3T3 cell line. Micrographs (Figure 1a) showed NIH-3T3 cell line in different doses of OB, with doses above 10 mg/mL showing visible effects on cell viability. In particular, cells incubated with OB till 5 mg/mL concentration still showed a high cell density (Figure 1a, upper land). To the OB concentration of 10 mg/mL, the viable cells drastically lowered (Figure 1a, lower land). Consistent with (Figure 1a), the percent of survived cell was over 70 percent in the range of 0 to 0.625 mg/mL. At OB dose of 1.25mg/mL, the ratio of live cell was approximately 50%. This ratio decreased dramatically to 15 percent at the dose of 10 mg/mL (Figure 1b). Based on this data, the IC_{50} (half maximal inhibitory concentration) of OB for NIH-3T3 cell line was 2.15 mg/ml and the LD_{50} (lethal dose 50%) calculated based on IC_{50} was approximately 1832 mg/kg.

In vivo acute and chronic toxicity on Swiss mice

Next, we investigated OB safety *in vivo* using Swiss mice. According to the LD_{50} calculated above, the guidelines for toxicity testing and physical properties of OB powder we decided to use the dose of 250 mg/kg/day in both acute and chronic toxicity tests.[20,21] For acute toxicity testing at the OB dose of 250 mg/kg/day, mice did not show any abnormal or fatal signs after 48h of oral administration. Therefore, it could be concluded that OB did not cause acute toxicity at 250 mg/kg/day.

For chronic toxicity testing of 30 days at 250 mg/kg/day, we did not observe any adverse effects in OB-treated mice. Specifically, there was no significant diffrence in the hematology formula between the control and OB-treated group, with the average amount of WBC, RBC and HGB in OB-treated group being $6.58\pm2.85.10^3$ cells/µL, $8.13\pm0.82.10^6$ cells/µL and 12.33±1.44 g/dL, respectively, compared to $6.18\pm1.58.10^3$/µL, $8.81\pm0.4.10^6$/µL and 14.13±0.59 g/dL in the control group (Figure 2, Supplement Table 1). In addition, the body weights also showed no difference between the two groups (Figure 3, Supplement Table 2). Therefore, the consumption of OB mucilage at 250 mg/kg/day was safe in all assays tested.

Lipid adsorption/absorption capability of mucilage from Ocimum basilicum using sulfo-phospho-vanillin colorimetric method

The ability to absorb/adsorb lipid from different lipid sources of OB mucilage was assessed by applying sulfo-phospho-vanillin method. The result indicated that OB mucilage was able to absorb/adsorb lipid from different types of fatty acid chain. Using 10 mg of OB dried

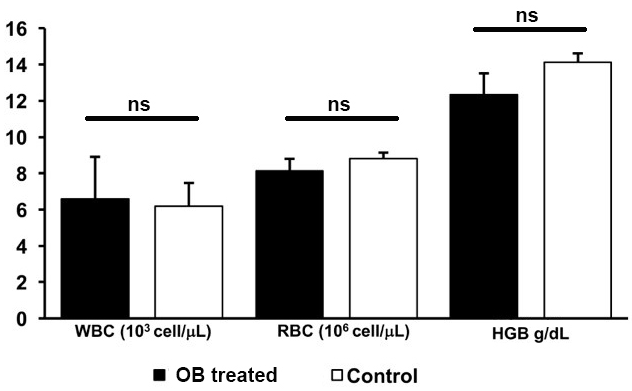


Figure 2: Haematology test after 30 days of chronic toxicity testing.

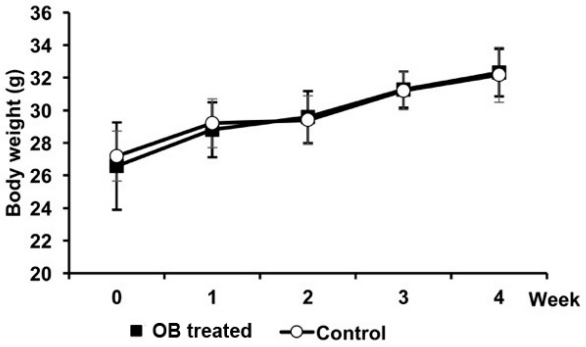
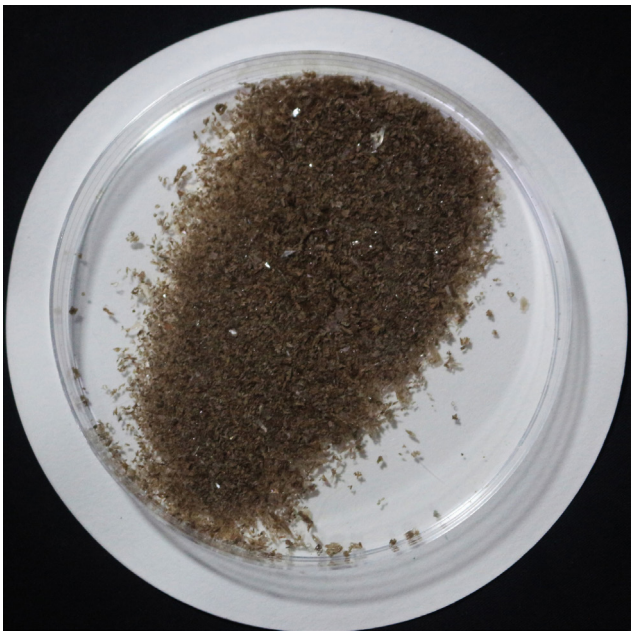


Figure 3: Body weight after 30 day of chronic toxicity testing.

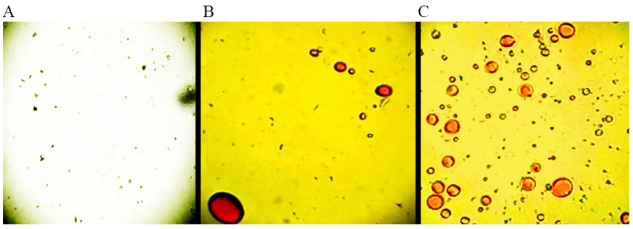


Supplement Figure 1: Dried mucilage texture and color.

mucilage, the absorbed/adsorbed lipid amount was 2.1 µg and 18.3 µg from coconut oil and butter, respectively (Supplement Table 3). To confirmed that, the OB mucilage was retrieved after incubation with oil, washed and used for direct quantification of lipid (data not shown) and stained for lipid entrapped by oil-red-O' reagent (Supplement Figure 2).

DISCUSSION

Obesity and related diseases are one of the top health issues that threaten our society. Due to the development of the fast food industry and the changing of modern lifestyle, treatments for overweight/obesity are becoming less effective than ever. To overcome this shortage, using anti-obesity drugs results in faster and



Supplement Figure 2: Oil-red-O' staining for lipid adsorption/ absorption ability of OB mucilage. (A), mucilage alone (control); (B), mucilage incubated with lipid from either coconut oil or butter; (C), lipid droplets (control).

Table 1S: The blood composition of mice groups after *in vivo* toxicity evaluation.

	OB mucilage treated			Control		
Indices	1	2	3	1	2	3
WBC (10^3/µL	5.16	9.86	4.72	4.67	6.04	7.82
RBC (10^6/µL)	9.05	7.48	7.86	9.09	8.99	8.35
HGB (g/dL)	14	11.5	11.5	13.7	14.8	13.9

Table 2S: The weight of each mouse group during *in vivo* toxicity evaluation.

		OB mucilage treated			Control		
		1	2	3	1	2	3
Weight (g)	Week 0	26.60	23.28	29.85	25.55	26.78	29.24
	Week 1	27.72	27.51	31.19	29.86	27.12	30.61
	Week 2	28.13	28.83	31.80	30.06	27.34	30.80
	Week 3	30.00	31.10	32.7	30.43	30.32	32.84
	Week 4	30.56	32.25	34.13	31.11	30.86	34.56

Table 3S: The ability of lipid absorption/adsorption of OB mucilage.

Lipid source	Concentration (mg/mL)	Absorbed/Adsorbed lipid concentration (µg/mL)			
		1st	2nd	3rd	Mean ± SD
Coconut oil	100	3.33	11.11	6.11	6.85±3.94
Butter	100	53.56	67.16	61.16	60.63±6.82

better treatment. However, treatments with drug have to cope with problems, which bring tremendous consequences, like safety dosage, side effect, etc. Therefore, utilizing nature, especially plants, derived products proven to be a good choice although their effects take longer time than drugs.

Basil (*Ocimum basilicum* L.) is a member of genus *Ocimum,* which comprises of up to 150 species of herbs and shrubs. It can be found in the tropical regions of Asia, Africa and Central and South America.[15] In Asia, basil seeds were frequently used in beverages and desserts for aesthetic purposes as well as a source of dietary fiber. It is also used in traditional medicine to treat colic ulcer, dyspepsia, diarrhea and inflammations. However, the numbers of proper studies on the effects of OB were very few. The uniqueness of OB seed comes from its mucilage which is a layer around the seed containing mainly of polysaccharide. When soaked in water, this layer swells and enables them to be used as a low energy gastric filler and fat absorber. Furthermore, its high polysaccharide content might be effective in controlling intestinal microbial composition. This work documented for the first time the biological effects of this mucilage layer; specifically, we showed that OB mucilage was well tolerated in mice models and its consumption was associated with positive benefits including weight control and promoting healthy intestinal microbial composition.

There were several methods to extract seed mucilage, which could be classified into using physical, chemical and combined physical and chemical methods.[16] By using solvents such as heptane/hexane, the yield of mucilage receiving could beyond 20% (data not shown). But for the purpose of developing a novel functional food in obesity controlling, using only water and physical forces was strictly required. Therefore, through stirring and filtering, we retrieved about 10% mucilage mass compared to the initial seed mass. Our extraction yield was as high as the yield of chia seed (*Salvia hispanica* L.) extraction reported by Tavares *et al*.[22] Research on other mucilage extraction showed a range of mucilage yield when obtaining the polysaccharide layer from several seeds such as *A. thaliana*, *L. usitatissimum*, *P. ovata* and *P. cunninghamii* from 4% to 23.8%.[23] Nazir *et al*. optimized the extraction methods for retrieving dried mucilage from *O. basilicum* L. as well and the reported yield was 20.5%.[24] Based on that, the seed mucilage extraction yield could be different depends on the plant's specie and the protocol could be optimized to reach a higher yield. The texture and characteristic of OB mucilage obtained also the same as described in those studies. The change in mucilage color might due to heat degradation of polysaccharide chains because of the drying temperature.[25] To overcome this problem, mucilage should be freeze dried to maintain the polysaccharide chain structure.

For further examining the effect of heat dry onto OB mucilage, we performed the reducing sugar measurement using DNS reagent. According to that, the concentration of reducing sugar of dried mucilage raised 37 times higher than the extracted polysaccharide. The raising of reducing sugars might increase the risk of sugar consumption therefore increase the risk of obesity, diabetes and other consequences.[26,27] For a high functioning obesity control product, this should be avoided, so that the optimization of drying condition should be evaluated.

To be considered as an appropriate functional food for obesity control, the bulk of volume/weight of dried mucilage was the first to be characterized. As in our result, the swelling index of OB mucilage reached 20 times higher than the dried weight and was stable in different pH solution. A research conducted on *O. basilicum* L., in which the swelling ratio was 1.1 times (11%),[28] much lower than that of basil seeds mucilage. Although mucilage derived from the same plant species, our mucilage possessed a much higher swelling ratio, suggesting a higher application range for *Ocimum basilicum* L. mucilage. Our result was quite the same as other seeds mucilage such as *Salvia hispanica* L. (swelling ratio of 27 times) and *Ocimumtenui florum* L. (swelling ratio of 20 times)[16,29] which suggested OB mucilage would also fit not only for functional foods but also for applications in drug release, food thickener.

Our study is the first study showed that OB mucilage was safe for consumption by detailed evaluations in both *in vitro* and *in vivo* assays, including cytotoxicity in NIH-3T3 cell line, acute and chronic toxicity in Swiss mice for up to 30 days, despite its widespread and regular used in Asian diets, this is the first systematic investigation of OB safety, defining a safe dose for further studies.

After proven safety on cells and animal, we evaluated the ability of OB mucilage to develop a natural weight control product via assessing the lipid absorption/adsorption capability. The result indicated that lipid droplets from either coconut oil or butter were trapped within OB's structure. There was a significant difference in the absorption/adsorption of the two kind of lipid source, which we could explain based on the characteristic of these two. Particularly, butter stays in its solid state at room temperature so when dissolved in water, lipid droplets were more likely formed. In contrast,

lipid from coconut oil is more common to accumulate into large molecules, which was hard to have stuck in the mucilage structure. Nonetheless, this result still supports the chance of OB mucilage for developing a functional food for weight control, since the rapid growth the fast food industry is hampering our healthy lifestyle.

CONCLUSION

In conclusion, our results showed for the first time that OB mucilage is a potential periodic for weight control, laying the groundwork for the development of OB-based functional foods to help control weight or reduce obesity.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

BMI: Body Mass Index; **CHD:** Coronary heart disease; **DMEM:** Dulbecco' Modified Eagle's Medium; **DMSO:** Dimethyl sulfoxide; **DNS:** Dinitrosalicylic acid; **HGB:** Hemoglobin; **OB:** *Ocimum basilicum* L. dried mucilage; **PBS:** Phosphate Buffered Saline; **RBC:** Red blood cells; **WBC:** White blood cells; **WHO:** World Health Organization.

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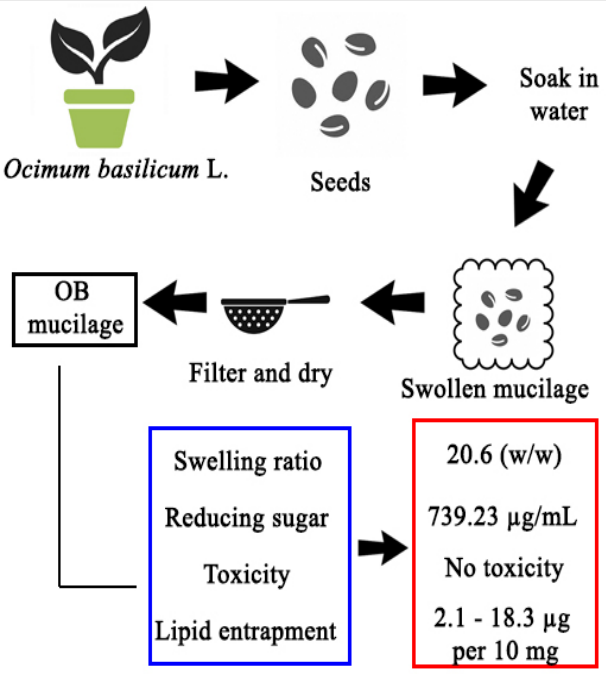
PICTORIAL ABSTRACT



SUMMARY

In this study, we extracted and evaluated several characteristics of mucilage from basil seed (*Ocimum basilicum* L.). The extraction yield was about 10%, with a slight change in the mucilage appearance. The dried mucilage has the swelling ratio of 20.6 (w/w) and considered stable at pH ranging from 1.5 to 6.5. The reducing sugar presented in dried mucilage was 739.23 µg/mL, 37 times higher than the raw mucilage. Dried mucilage isolated from *Ocimum basilicum* L. (OB) shows no toxicity both *in vitro* and *in vivo* with the lethal dose of 1.8 g/kg body weight/day. Besides, OB mucilage spossessed the ability to absorb/adsorb 2.1 and 18.3 µg of coconut oil and butter, respectively, per 10 mg of dried mucilage.

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