Songklanakarin J. Sci. Technol.
40 (5), 1076-1080, Sep. - Oct. 2018

http://www.sjst.psu.ac.th

Original Article

Mucilage powder from *Litsea glutinosa* leaves stimulates the growth of cultured human hair follicles

Worapan Sitthithaworn[1*], Mattaka Khongkaw[2], Chutima Wiranidchapong[3], and Thongchai Koobkokkruad[2]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhon Nayok, 26120 Thailand*

[2] *National Nanotechnology Center, National Science and Technology Development, Khlong Luang, Pathum Thani, 12120 Thailand*

[3] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhon Nayok, 26120 Thailand*

Received: 2 November 2016; Revised: 2 May 2017; Accepted: 28 June 2017

Abstract

The cleaning property of mucilage from the leaves of *Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae) and its ability to promote hair growth were investigated. Mucilage was obtained by boiling dried leaves and was spray dried to result in the powder form. The cleaning property was investigated by examining the surface tension of the mucilage using a du Noüy ring tensiometer. Mucilage powder appeared to reduce water surface tension to a minimum value of 44.95 mN/m when the concentration of mucilage was increased to 20% (v/v) (equivalent to 460 µg/ml). A similar result was observed from mucilage in its original liquid form. The hair growth modulating effect of mucilage was determined on human hair follicle dermal papilla (HFDP) cell lines using the MTT assay. Prominent cell proliferation was observed at a concentration of 250 µg/ml of mucilage after 24 h in culture (a 1.4-fold increase compared to the control).

Keywords: *Litsea glutinosa*, mucilage, surface tension, human hair follicles

1. Introduction

Litsea glutinosa (Lour.) C.B. Robinson (Lauraceae) (Figure 1) is an evergreen tree distributed throughout South-eastern Asia. In Thailand, it is abundant in the northern and northeastern parts of the country. The leaves contain essential oil, of which beta-ocimene and caryophyllene are major constituents (Nguyen *et al.*, 2010). The leaves and bark are mucilaginous. It was reported that the water-soluble polysaccharide arabinoxylan can be isolated from the mucilaginous substance (Dasa *et al.*, 2013; Herath *et al.*, 1990). The leaves of *L. glutinosa* are used as an ingredient to make traditional hair shampoos due to their mucilaginous nature. It is

Figure 1. The mature leaves of *Litsea glutinosa* (Lour.) C. B. Robinson (Lauraceae).

*Corresponding author
Email address: worapan@g.swu.ac.th

believed that mucilage from the leaves helps clean the hair and promotes hair growth. To make hair shampoo, the leaves are either squeezed or cut and boiled to obtain the mucilage and then mixed with other herbs such as cowpea flower (*Critoria ternatea*, Leguminosae) and juice of leech lime (*Citrus hystrix*, Rutaceae) fruit. These methods are used in household remedies and in small-scale enterprises. Mucilage in its liquid form is likely prone to microbial contamination and can be difficult to store and transport, making it a barrier for large-scale production. Spray drying has recently been used to efficiently produce a dry powder from a liquid by immediately drying with hot air (Mujumdar, 2014). Altering mucilage to its dry powder form would provide the advantages of preservation as well as weight and volume reduction.

In addition, the cleaning property of mucilage from *L. glutinosa* leaves has never been demonstrated scientifically. The cleaning property can be characterized by an ability to lower water surface tension, thus enabling the water to spread more and penetrate pores in the surface. Mucilage from *L. glutinosa* leaves is hypothesised to reduce water surface tension. To this end, mucilage samples were obtained by conventional methods, i.e., squeezing of fresh leaves or boiling of dried leaves. Mucilage obtained from the squeezing of fresh leaves was coded as M-F, and the mucilage obtained from the boiling of dried leaves was coded as M-B. Both M-F and M-B were investigated for their abilities to reduce water surface tension using a du Noüy ring tensiometer. To determine whether mucilage can be altered to powder form without changing its ability to reduce water surface tension, one half of M-B was further converted into a powder using the spray drying method and was coded as M-P. M-P was reconstituted and examined for its ability to reduce water surface tension.

Furthermore, to evaluate its possible influence on hair growth, the blue tetrazolium reduction (MTT) assay was applied to investigate the growth modulating effect of mucilage in its powder form on a cultured human hair follicle cell line.

2. Materials and Methods

2.1 Plant materials

The mature leaves of *Litsea glutinosa* (Lour.) C.B. Robinson were collected from the botanical garden of the Faculty of Pharmacy, Srinakharinwirot University, Nakonnayok, located in central Thailand. A voucher specimen (no. ws0124) was deposited in the herbarium of the same institute.

2.2 Extraction of mucilage

Mucilage was extracted from mature *Litsea glutinosa* (Lour.) C.B. Robinson leaves using three methods: 1) boiling of dried leaves, 2) boiling of dried leaves and then converting to dried powder using a spray dryer, and 3) squeezing of fresh leaves. For the boiling method, leaves (200 g) were dried (40°C) in a hot air oven and then converted into powder using a cutting mill (Pulverisette 15, Fritsch GmbH, Germany), before boiling in deionized water (2,000 ml) for 1 h. To obtain mucilage from fresh leaves, leaves (100 g) and deionized water (1,000 ml) were mixed and squeezed by hand. The mucilage was separately filtered and the leaves were taken in four layers of muslin cloth and squeezed to remove the remaining mucilage. The mucilage obtained from the boiling method was divided into two equal parts. The first part was maintained in a deep freezer (-20°C) for further studies; this sample was coded as M-B. The other part was subjected to a spray drying to remove water. The powder weighed 2,300 mg after water was removed and was coded as M-P. The mucilage obtained by the squeezing of fresh leaves was coded as M-F.

2.3 Spray drying conditions

A Buchi mini spray dryer B-290 (Buchi labortechnik AG, Flawil, Switzerland) was used to remove water from the mucilage. The conditions were as follows: inlet temperature 155°C, outlet temperature 122°C, feed rate of 5 ml/min, air flow rate of 30 m^3/h, and spray flow rate of 473 l/h.

2.4 Determination of surface tension

Mucilage in its powder form (M-P) was reconstituted to make the concentration equivalent to the original liquid mucilage. The powder obtained from liquid mucilage (1,000 ml) was 2,300 mg. Therefore, after wetting with ethanol (10 ml), M-P (230 mg) was dispersed in deionized water (90 ml). Thus, deionized water with ethanol at a concentration of 10% (v/v) was used to make the mucilage dilutions. To determine the surface tension, various concentrations of M-F, M-B, and M-P were measured using the du Noüy ring tensiometer (Tensiometer K 6 (KRUSS), Germany). The experiment was performed at 25°C. A platinum wire ring was immersed in the samples. The distance between the immersed ring and the liquid surface was fixed at 5.0 mm to ensure a clean break of the meniscus on the immersed platinum ring. The immersed ring was pulled slowly through the liquid-air interface, where the tensiometer platform was moved in the opposite direction at the same time. The radius (R) of the ring and the ring dimensions ratio (R/r) were given by the manufacturer as 9.545 mm and 51.6, respectively. The surface tension value and the correction factor were, respectively, calculated according to Equation 1 and the mathematical model proposed by Zuidema and Waters (1941), as displayed in Equation 2.

$$\gamma = f/4\pi R \quad (1),$$

$$\beta = 0.725 + \sqrt{\{0.01452 f/4\pi^2 R^2(\rho_1 - \rho_2)\} + 0.04534 - 1.679/(R/r)} \quad (2)$$

where γ is the surface tension (mN/m); f is the maximum pull force (mN); R is the radius of the du Noüy ring (mm); β is the correction factor; ρ_1 and ρ_2 are the densities of the mucilage dispersion and air, respectively; and R/r is the ring dimensions ratio.

The calibration was performed using distilled water (surface tension = 72.0 mN/m ± 0.5). Between each measurement, the platinum ring was rinsed three times with distilled water and then burned to clean and dry. The surface tension results were taken five times from the same concentration and data were presented as mean ± standard error (SE). To com-

pare each group, one-way analysis of variance (ANOVA) was performed using SPSS program for Windows, version 24.0 (SPSS Inc., Chicago, USA). Significant differences between means were determined by Scheffe's test. Statistical significance was defined as $P < 0.05$.

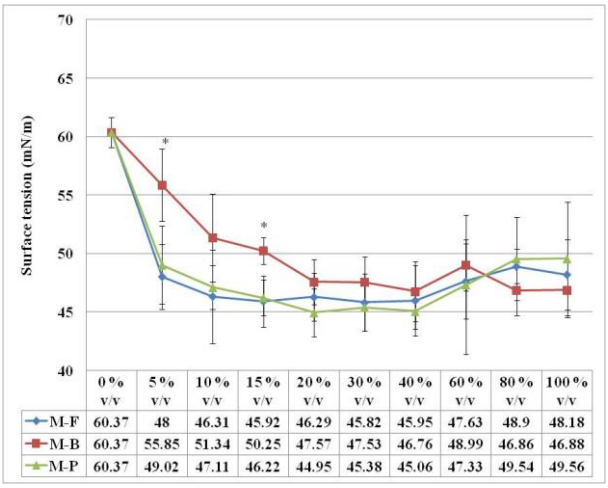
2.5 Cell proliferation assay

Human hair follicle dermal papilla (HFDP) cell lines were purchased from the American type culture collection (Virginia, USA). The growth modulating effects of mucilage samples on HFDP cells were determined by the MTT assay (Carmichael *et al*., 1987). One hundred microliters of 1×10^4 cells was plated into each well of a 96-well plate and maintained in culture for 24 h in a humidified atmosphere (37˚C, 5% CO_2). After incubation, the cells reached 80% confluency. The medium was removed and cells were incubated in the presence of the spray-dried mucilage sample diluted in DMSO in a series of two-fold dilutions varying from 4 µg/ml to 1,000 µg/ml for 24, 48, and 72 h at 37°C. After removal of the sample solution, 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazoliumbromide (MTT) in phosphate buffer was added. After a 4-h incubation, MTT solution was discarded and 100 µl DMSO were added. Viable cells were determined by measuring the absorbance at 570 nm. The effect of the samples on proliferation is expressed as the % cell viability using the following formula: % cell proliferation = [absorbance of the treated sample/absorbance of the untreated sample]x100. Minoxidil was used as positive control. Data are presented as the mean values ± standard deviation calculated from triplicate experiments. Student's t-test was used to determine the statistical significance ($P < 0.05$) of the differences between the experimental and control groups.

3. Results and Discussion

The leaves of *L. glutinosa* have been used as a hair cleaner and hair tonic since ancient times in Thailand. In this study, mucilage was obtained using traditional processes by either squeezing of fresh leaves to obtain M-F or by boiling of dried leaves to obtain M-B. However, liquid mucilage was difficult to handle. Therefore, the spray drying method was applied to remove water from M-B. After the spray drying process, the mucilage yielded 0.23% (w/v) of dry M-P powder. The M-P appeared as yellowish-white fine powder.

The cleaning property of mucilage from the leaves of *L. glutinosa* was investigated by examining the surface tension of M-F, M-B, and M-P using the du Noüy ring tensiometer. The M-P powder (230 mg) was converted into liquid form by wetting with ethanol (10 ml) before dissolving in deionized water (90 ml). Thus, deionized water with ethanol at a final concentration of 10% (v/v) was used to prepare the dilutions of mucilage solutions. The surface tension of 10% (v/v) ethanol in deionized water determined in this study was 60.37 mN/m; this value was between the surface tension of deionized water (72 mN/m ± 0.5) and pure ethanol (22 mN/m ± 0.5) (Vargaftik *et al.*, 1983). It was apparent that M-P reduced the surface tension of water at every concentration tested (Figure 2). Similar results were also observed using M-F and M-B. ANOVA test revealed that surface tension compared at each concentration between M-P



	0% v/v	5% v/v	10% v/v	15% v/v	20% v/v	30% v/v	40% v/v	60% v/v	80% v/v	100% v/v
M-F	60.37	48	46.31	45.92	46.29	45.82	45.95	47.63	48.9	48.18
M-B	60.37	55.85	51.34	50.25	47.57	47.53	46.76	48.99	46.86	46.88
M-P	60.37	49.02	47.11	46.22	44.95	45.38	45.06	47.33	49.54	49.56

Figure 2. Surface tension of mucilage at the concentrations 0-100% (v/v). Error bars indicate standard error from five measurements. Statistical significance between means of M-F, M-B and M-P was determined by one-way analysis of variance (ANOVA) followed by Scheffe's test. Statistical significance was defined as *, $P < 0.05$.

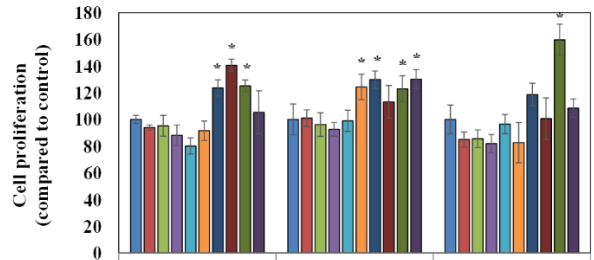
and M-F was not significantly different while M-B at the concentration of 5% (v/v) and 15% (v/v) was significantly different from M-F and M-P. Notably that the surface tension at the critical micellar concentration (CMC) among M-B, M-F and M-P was exhibited to be the same. The CMC was observed when the mucilage reached a concentration of 20% (v/v), which was equivalent to 460 µg/ml of M-P when it was redispersed. The surface tension of the mucilage at the CMC ranged between 47.57 mN/m and 44.95 mN/m. These results suggest that the mucilage obtained from *L. glutinosa* leaves obtained from either the squeezing of fresh leaves, the boiling of dry leaves or the spray dried powder possessed cleaning properties since they each lowered the water surface tension. In addition, we conclude that mucilage could be converted into powder using the spray drying method without changing its surface-active property.

Mucilage from the leaves of *L. glutinosa* is mainly composed of water-soluble polysaccharides (Dasa *et al.*, 2013); water-soluble polysaccharides are substances such as biopolymers that tend to accumulate at the surface rather than remaining in the bulk when they are dissolved in water. (Docoslis *et al.*, 2000; Morrison & Ross, 2002). This behaviour is similar to that of a surface active agent that is commonly used as an active ingredient in cleaning products. As a result, we concluded that the apparent reduction in water surface tension observed in this study as the mucilage concentration increased up to CMC and lack of any further decrease when the mucilage concentration increase was due to surfactant properties.

A small increase in surface tension when the mucilage concentration was higher than 20% (v/v) was a result of an increase in viscosity. This typically influenced water surface tension when measured using the du Noüy ring tensiometer (Lee *et al.*, 2012) because only a portion of the liquid raised by the ring broke away from the surface and attached to the ring, whereas a smaller portion of it remained

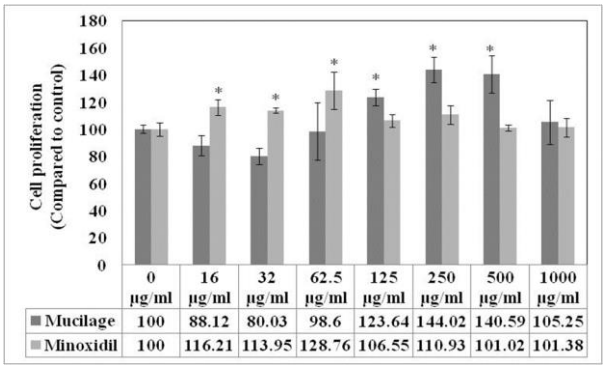
undetached from the surface. This problem could cause an error of 30% or more on the measured pull force (*f*) required to detach a circular ring from the surface of the liquid sample (Huh & Mason 1975).

The results revealed that the mucilage could be converted into powder without altering its surface-active property. In addition, the mucilage in powder form is more favorable for processing on an industrial scale because it is easy to store and transport. To determine whether M-P could promote hair growth, M-P was applied to human hair follicle dermal papilla (HFDP) cell lines. After treatment with different concentrations of M-P, HFDP cells were assayed for cell proliferation. The results revealed that M-P stimulated cell growth prominently after 24 h in culture (Figure 3). The percent of cells metabolizing MTT after 24 h in culture versus the control was +23%, +40%, and +25% at 125 µg/ml, 250 µg/ml, and 500 µg/ml, respectively. The growth-modulating effect was prolonged to 48 h in culture and declined when cells were exposed to mucilage for a longer period of time. Minoxidil was also tested for cell proliferation assay and it was found that minoxidil could enhance cell proliferation at low concentrations (16 µg/ ml, 32 µg/ ml and 62. 5 µg/ ml) while higher concentrations of M-P were required for stimulate cell proliferation to reach cell number equivalent to that performed by minoxidil (Figure 4). Minoxidil is a chemical approved by the US Food and Drug Administration to treat hair loss (Boisvert *et al*., 2017). These results suggest that mucilage from *L. glutinosa* leaves has the potential to modulate hair growth in humans.



	24h	48h	72h
0 µg/ml	100.0	100.0	100.0
4 µg/ml	93.9	101.0	85.0
8 µg/ml	95.2	96.1	85.6
16 µg/ml	88.1	92.6	81.8
32 µg/ml	80.0	98.9	96.5
62.5 µg/ml	91.5	124.3	82.6
125 µg/ml	123.6	129.8	118.6
250 µg/ml	140.6	113.2	100.6
500 µg/ml	125.1	123.0	159.6
1000 µg/ml	105.2	130.2	108.5

Figure 3. Growth modulating effects of mucilage in HFDP cells. HFDP cells were treated with mucilage (M-P) solution for 24, 48, and 72 h. Cell proliferation was monitored by the MTT assay. The percentage of proliferation was calculated using the following formula: (proliferated cells) % = (absorbance of the treated sample/ absorbance of the untreated sample) x 100. Values are reported as the mean ± SD. n = 3. Statistical significance was determined by Student's t-test (*, $P \leq 0.05$ versus control).



	0 µg/ml	16 µg/ml	32 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Mucilage	100	88.12	80.03	98.6	123.64	144.02	140.59	105.25
Minoxidil	100	116.21	113.95	128.76	106.55	110.93	101.02	101.38

Figure 4. Growth modulating effects of mucilage and minoxidil in HFDP cells. HFDP cells were treated with mucilage (M-P) or minoxidil for 24 h. Cell proliferation was monitored by the MTT assay. The percentage of proliferation was calculated using the following formula: (proliferated cells) % = (absorbance of the treated sample/absorbance of the untreated sample)x100. Values are reported as the mean ± SD. n = 3. Statistical significance was determined by Student's t-test (*, $P \leq 0.05$ versus control).

The hair growth effects of mucilage from the leaves of *L. glutinosa* may be due to the polysaccharide arabinoxylan, which is found in mucilage (Dasa *et al.*, 2013; Herath *et al.*, 1990). Mucilage may provide nutrients for hair follicles. Polysaccharides from several plants have been reported to promote hair growth. For example, polysaccharides from *Dendrobium candidum* have been proven to promote hair growth in mice (Chen *et al.*, 2014). A fraction of polysaccharides from *Lycium barbarum*, a traditional Chinese herbal medicine, have exhibited positive effects on cisplatin-induced hair cell damage in organ of Corti explants (Liu *et al*., 2011). Apart from plant polysaccharides, several herbal extracts with water have been reported to increase the growth of HFDP cells. For example, *Asarum sieboldii* or asiasari radix extract has been reported to increase the growth of HFDP cells via enhancing the expression of vascular endothelial growth factor (Rho *et al.*, 2015). Recently, a combination of extracts from the fruits of *Persea americana*, flowers of *Althaea officinalis*, *Chamaemelum nobile*, and *Thymus vulgaris*, and leaves of *Rosmarinus officinalis* and *Urtica dioica* have also been demonstrated the ability to promote the growth of HFDP cells by regulating the expression of several genes involved in cell proliferation (Rastegar *et al.*, 2015) . In addition, ethanolic extract of *Catharmus tinctorius* which is traditionally used in Thailand for hair nourishment has been shown to promote hair growth by inhibiting the 5α-reductase activity isolated from rat livers (Kumar *et al.*, 2012). In another experiment, it was found that a flavonoid, hydroxysafflor yellow A in *C. tinctorius* was responsible for hair growth by induction of hair growth-associated gene expression in HFDP cells (Junlatat & Sripanidkulchai, 2014). Although several plant extracts were reported to promote hair growth, organic solvent was used in order to obtain the desired compounds and the organic solvent must be removed before processing to the formulation step. The mucilage has the benefit over chemical extract because it could be obtained using water. Thus, it is safe to use in household remedies and in small-scale enterprises.

4. Conclusions

The results of this study demonstrated the ability of mucilage to lower the surface tension of water as well as its potential to enhance the proliferation of human HFDP cells. Mucilage could be spray-dried without changing its surface-active property whilst retaining its hair growth promotion property. Thus, mucilage from the leaves of *L. glutinosa* is a promising active ingredient for hair care formulations. These results also provided scientific evidence to support the traditional use of *L. glutinosa* leaves for cleaning hair as well as for hair growth promotion. In order to achieve a potential active ingredient for hair care formulation, the active constituent of the mucilage should be clarified. In addition, *in vivo* study of the hair growth promoting effect of the mucilage in animal such as study of the re-growth rate of shaved-animal hair should be performed. Moreover, during the *in vivo* study, toxicity studies, such as irritation, the differences in average body weight between treatment and non-treatment groups and abnormalities in the animal should be observed.

Acknowledgements

The authors would like to thank the Research Unit for Drug Discovery and Development, Faculty of Pharmacy, Srinakharinwirot University, for providing laboratory facilities. This work was financially supported by the Office of the Higher Education Commission (Grant No. 216/2558).

References

Boisvert, W. A., Yu, M., Choi, Y., Jeong, G., Zhang, Y., Cho, S., . . . Lee, B. (2017). Hair growth-promoting effect of *Geranium sibiricum* extract in human dermal papilla cells and C57BL/ 6 mice. *BMC Complementary and Alternative Medicine, 17*, 109-118.

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., & Mitchell, J. B. (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Research, 47*, 936-942.

Chen, J., Qi, H., Li, J. B., Yi, Y. Q., Chen, D., Hu, X. H., . . . Wei, X. Y. (2014). Experimental study on *Dendrobium candidum* polysaccharides on promotion of hair growth. *Zhongguo Zhong Yao Za Zhi, 39*(2), 291-295.

Dasa, D., Maitib, S., Maitib, T., & Islama, S. (2013). A new arabinoxylan from green leaves of *Litsea glutinosa* (Lauraeae): Structural and biological studies. *Carbohydrate Polymers, 92*, 1243-1248.

Docoslis, A., Giese, R. F., & Van Oss, C. J. (2000). Influence of the water-air interface on the apparent surface tension of aqueous solutions of hydrophilic solutes. *Colloids and Surfaces B: Biointerfaces, 19*, 147-162.

Herath, H. M., Kumar, N. S., & Wimalasiri, K. M. (1990). Structural studies of an arabinoxylan isolated from *Litsea glutinosa* (Lauraceae). *Carbohydrate Research, 198*, 343-351.

Huh, C., & Mason, S. G. (1975). A rigorous theory of ring tensiometry. *Colloid and Polymer Science, 253*, 566-580.

Junlatat, J., & Sripanidkulchai, B. (2014). Hair growth-promoting effect of *Carthamus tinctorius* floret extract. *Phytotherapy Research, 28*, 1030-1036.

Kumar, N., Rungseevijitprapa, W., Narkkhong, N. A., Suttajit, M., & Chaiyasut, C. (2012). 5α-reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment. *Journal of Ethnopharmacology, 139*, 765-71.

Lee, B. B., Chan, E. S., Ravindra, P., & Khan, T. A. (2012). Surface tension of viscous biopolymer solutions measured using the du Noüy ring method and the drop weight methods. *Polymer Bulletin, 69*, 471-489.

Liu, Q., Li, Y., Hu, L., & Wang, D. (2011). *Lycium barbarum* polysaccharides attenuate cisplatin-induced hair cell loss in rat cochlear organotypic cultures. *International Journal of Molecular Sciences, 12*, 8982-8992.

Morrison I. D., & Ross, S. (2002). *Colloidal dispersions, suspensions, emulsions, and foams*. New York, NY: John Wiley and Sons.

Mujumdar, A. S. (2014). *Principles, classification, and selection of dryers. In handbook of industrial drying*. New York, NY: CRC Press.

Nguyen, T. H., Tran, D. T., Do, N. D., & Tran, H. T. (2010). Chemical composition of the leaf oil of *Litsea glutinosa* (Lour.) C. B. Rob. from Ha Tinh province. *Vietnam National University Journal of Science. (Natural Sciences and Technology), 26*, 161-164.

Rastegar, H., Ashtiani, H. A., Aghaei, M., Barikbin, B., & Ehsani, A. (2015). Herbal extracts induce dermal papilla cell proliferation of human hair follicles. *Annals of Dermatology, 27*(6), 667-675.

Rho, S. S., Park, S. J., Hwang, S. L., Lee, M. H., Kim, C. D., Lee I. H., . . . Rang M. J. (2005). The hair growth promoting effect of Asiasari radix extract and its molecular regulation. *Journal of Dermatological* Science, *38*, 89-97.

Vargaftik, N. B., Volkov, B. N., & Voljak, L. D. (1983). International tables of the surface tension of water. *Journal of Physical and Chemical Reference Data, 12*(3), 817-820.

Zuidema, H., & Waters, R. G. (1941). Ring method for the determination of interfacial tension. *Industrial and Engineering Chemistry, 13*, 312–313.