Evaluation of the hair growth promotion effect of *Broussonetia papyrifera* bark extract

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Introduction:

Hair is an organ composed of the multi-layered structure of cuticle, cortex, and medulla. Hair protects the head and scalp from ultraviolet rays, heat, and physical shock [1]. Hair grows from the root of hair follicles, components of mesenchymal-epithelial interaction. Hair follicles repeat growth and degeneration according to the growth cycle of anagen (growth phase), catagen (regression phase), and telogen (resting phase) [2]. The growth phase and resting phase maintain a 9:1 ratio to control the number of hairs. If the ratio changes or the hair follicles are degenerated, the hair falls out excessively resulting in hair loss, also known as alopecia [3].
Wnt/β-catenin signaling and JAK/STAT signaling are signals involved in hair growth, and play important roles in regulating the development and growth cycle of hair follicles, respectively [4, 5]. Therefore, assuming that the substances affecting these two signaling pathways will also affect the hair loss, *in vitro* studies and a clinical study on *Broussonetia papyrifera* bark extract were conducted to evaluate the hair growth promotion effect.

Materials & Methods:

Materials

In vitro study

B. papyrifera bark extract was extracted with 70% alcohol, freeze-dried, and diluted in DMSO (Dimethyl sulfoxide). Dermal papilla cells, NIH3T3 cells transformed with TCF/LEF-luciferase and HEK293 cells transformed with STAT6-luciferase were cultured in DMEM (Dulbecco's modified eagle medium) containing FBS (fetal bovine serum) and proper antibiotics.

Clinical study

Hair tonic was prepared by mixing and stirring the *B. papyrifera* bark extract with water and ethanol to a final concentration of 0.5%.

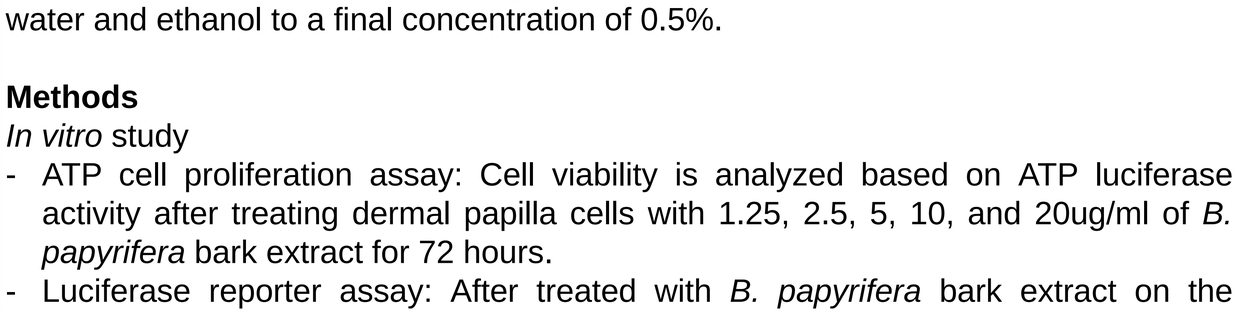
Methods

In vitro study

- ATP cell proliferation assay: Cell viability is analyzed based on ATP luciferase activity after treating dermal papilla cells with 1.25, 2.5, 5, 10, and 20ug/ml of *B. papyrifera* bark extract for 72 hours.
- Luciferase reporter assay: After treated with *B. papyrifera* bark extract on the transformed NIH3T3 cells and HEK293 cells, the activities of TCF/LEF and STAT6, which are the reporter gene of Wnt/β-catenin and JAK/STAT signaling were analyzed.
- RNA isolation and Real-time PCR: Total RNA was isolated after treated with *B. papyrifera* bark extract in dermal papilla cells and was amplified with β-catenin, TCF/LEF and STAT protein primers to quantify mRNA.
- Western blot: After treating *B. papyrifera* bark extract on dermal papilla cells, the cells were lysed to extract proteins from the supernatant and electrophoresis to quantify β-catenin, STAT6, phosphorylated β-catenin, and phosphorylated STAT6.

Clinical study

- 11 adults diagnosed with androgenetic alopecia applied hair tonic containing 0.5% *B. papyrifera* bark extract for 12 weeks daily. Phototrichogram and visual assessment were conducted to evaluate hair counts and hair distribution.

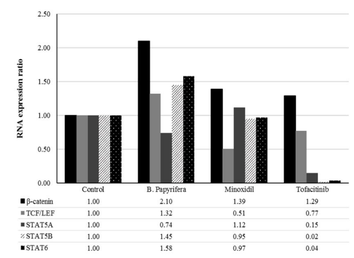


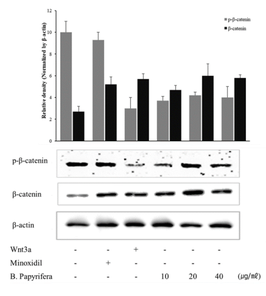
Results & Discussion:

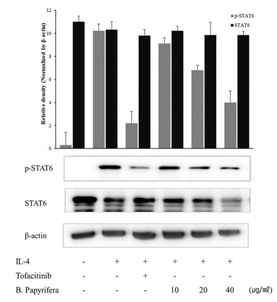
Results

In vitro study

- Promotion of dermal papilla cell growth: Compared to DMSO treated negative control, cell growth was significantly promoted when *B. Papyrifera* bark extract was treated at a concentration of 2.5, 5, 10, and 20ug/ml. Especially in high concentration, the effect was similar to that of minoxidil treated positive control.
- Regulation of hair growth-related signaling pathway: *B. papyrifera* bark extract treated NIH3T3 cells showed significant increase in TCF/LEF luciferase level compared to DMSO control. When JAK/STAT-activated HEK293 cells were treated with *B. papyrifera* bark extract, STAT6 luciferase was statistically decreased when the concentration was 10ug/ml or higher. Results of the RNA isolation and Real-time PCR, the activities of β-catenin and TCF/LEF, which involve in Wnt/β-catenin signaling pathway, increased compared to DMSO control when treated with *B. papyrifera* bark extract.
- Phosphorylation of β-catenin and STAT6: In dermal papilla cells, when treated with *B. papyrifera* bark extract, both phosphorylated β-catenin and phosphorylated STAT6 tended to decrease, suggesting that Wnt/β-catenin signaling was activated and JAK/STAT signaling was inhibited.







Clinical study

- The number of hairs of the hair loss area statistically significantly increased after application of hair tonic containing 0.5% of *B. papyrifera* bark extract for 12 weeks compared to before use.

Hair counts (n/cm²)	Baseline	6 weeks after	12 weeks after
Average	134.36	135.00	136.00
Standard deviation	25.453	26.234	26.412
Standard error	7.674	7.910	7.964
p-value		***0.490***	***0.008****

Discussion

B. papyrifera bark extract promoted the growth of dermal papilla cells and the promoting effect was similar to that of minoxidil, a hair loss treatment. Results of luciferase reporter assay showed that the expression of TCF/LEF, a transcription factor of Wnt/β-catenin signaling, increased in *B. papyrifera* bark extract treated group, whereas the expression of STAT6, a transcription factor of JAK/STAT signaling, decreased. Also, results of RNA isolation and Real-time PCR showed similar tendency of increased expression of β-catenin and TCF/LEF. In addition, both phosphorylated β-catenin and phosphorylated STAT6 decreased when treated with *B. papyrifera* bark extract. β-catenin is phosphorylated when the Wnt/β-catenin signaling is inactivated, so decrease of β-catenin phosphorylation could mean activation of Wnt/β-catenin signaling.

Conclusions:

Broussonetia papyrifera bark extract promotes the growth of dermal papilla cells and activates Wnt/β-catenin signaling by inhibiting β-catenin phosphorylation. As the results of clinical study showed effects of alleviating hair loss symptoms, *B. papyrifera* bark extract is expected to be used as a material for hair care products.

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References:

1. Yang, F. C., Zhang, Y., & Rheinstädter, M. C. (2014). The structure of people's hair. *PeerJ*, 2, e619.
2. Paus R. (1998). Principles of hair cycle control. *The Journal of dermatology*, 25(12), 793–802.
3. Lolli, F., Pallotti, F., Rossi, A., Fortuna, M. C., Caro, G., Lenzi, A., Sansone, A., & Lombardo, F. (2017). Androgenetic alopecia: a review. *Endocrine*, 57(1), 9–17.
4. Sennett, R., & Rendl, M. (2012). Mesenchymal-epithelial interactions during hair follicle morphogenesis and cycling. *Seminars in cell & developmental biology*, 23(8), 917–927.
5. Kishimoto, J., Burgeson, R. E., & Morgan, B. A. (2000). Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes & development*, 14(10), 1181–1185.