Pharmaciana
Vol. 8, No.1, May 2018, Page. 33-42
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v8i1.8877

Formulation and test activities of hair tonic with a combination of the aqueous extracts of aloe vera (*Aloe vera* L.) and licorice (*Glycyrrhiza glabra* L.)

Sulistiorini Indriaty[*1], Teti Indrawati[2], Shelly Taurhesia[2]
[1]Sekolah Tinggi Farmasi Muhammadiyah Cirebon
Jl. Cideng Indah no. 3, kertawinangun, Cirebon
[2] *Fakultas Farmasi University Pancasila*
Jl. Srengseng sawah, Jagakarsa, Jakarta

Submitted: 05-02-2018 ***Reviewed: 09-03-2018*** ***Accepted: 01-05-2018***

ABSTRACT

Aloe vera contains lignin, folic acid, vitamin A, and 20 types of amino acids, while *Glycyrrhiza glabra* L. contains glycyrrhizin, steroids, and beta-glycyrrhetinic acid that benefit for hair health. This research aimed to produce hair tonic with the best activity from the combination of aloe vera and licorice extract. The phytochemicals in the aqueous extract of licorice and *Aloe vera* were screened. The hair tonic was then prepared with 3 formulas using 7.5% *Aloe vera* extract and 2.5% licorice extract, as well as 96% ethanol with varying percentages, namely 5, 10, and 15%. Afterward, the formulas were tested for their activity as hair fertilizer on 4 white male rabbits for 28 days. The evaluation of hair tonic preparation included organoleptic observation, pH, specific weight, viscosity, and hedonic tests. It measured hair growth rate, the acceleration of hair growth, and hair weight. The results showed that hair tonic containing a mixture of 7.5% of Aloe vera extract, 2.5% of licorice extract, and 96% ethanol with different percentages (i.e., 5, 10, and 15%) created a good hair fertilizer. Hair tonic prepared with 15% of 96% ethanol exhibited an optimum activity. It was characterized by brown color, distinctive odor, homogeneity, a specific weight of 1.0577 g/mL, a viscosity of 2.8479 cP to distilled water, and pH 5.5. Its effectiveness as hair fertilizer was evidenced by a hair growth rate of 2.6617 cm in 28 days, a hair growth acceleration of 0.1635 cm/day, and a hair weight of 0.1854 gram in 28 days.

Keywords*: Aloe vera* L., *Glycyrrhiza glabra* L., hair tonic, hair growth rate, hair weight.

Corresponding author:
Sulistiorini Indriaty
Sekolah Tinggi Farmasi Muhammadiyah Cirebon
Jl. Cideng Indah no. 3, kertawinangun, Cirebon
Email: lovely_kalila@yahoo.co.id

INTRODUCTION

Hair tonic is a cosmetic dosage form used to promote hair growth in baldness or hair loss (Anonymous, 1985). Its application is allowed only for preventing hair loss and thinning, itchy scalp, and dandruff (Mitsui, 1996). Hair growth and care dosage forms are abundantly available in the market. Chemicals, which can give side effects to health, are still widely used as the active components even though there are many potential natural ingredients for stimulating hair growth. Many plants have the properties as hair fertilizer, such as *Glycyrrhiza glabra* L. (licorice) and *Aloe vera* L.

Aloe vera has been used to treat hair loss and to thicken and blacken hair since tens or even hundreds of years ago (Rostita, 2008). It contains ideal ingredients for cosmetics and dermatological products. It is now one of the essential components in cosmetics industry (Dutt, 2002). The five-percent hydroalcoholic extract of *Aloe vera* exhibits better activity of increasing hair growth and thickness in rats compared to negative control (base) when applied for one month (Jain, 2011).

Licorice is an ingredient used as hair grower. In Ayurveda, it is used as a beauty product for skin regeneration. Compared to minoxidil and negative control, the 2% petroleum ether extract of licorice results in longer hair growth in female rats when applied once daily for 30 days. The initial phase of hair growth occurred 5-13 days faster than with minoxidil. At the anagen state (the active phase of hair growth), this extract grows a maximum of 76% hair follicle, while treatments with minoxidil and control groups produce 66% and 45%, respectively (Sukirti, 2012). This research aimed (1) to create hair tonic with the best activity from the combination of *Aloe vera*, licorice, and different portions of 96% ethanol and (2) to evaluate the stability of hair tonic as a dosage form. It is expected to provide benefits and reference for similar studies and the development of other dosage forms.

MATERIALS AND METHODS

The tools used in this research were Ostwald viscometer, pH meter (Toledo), analytical balance (AND HR 20), caliper, hair shaver, and glassware. The materials included the aqueous extract of *Aloe vera* in powder dosage form (Java plant), the aqueous extract of licorice in powder dosage form (Java plant), menthol (Pro Technical CV. Mustika Lab), methylparaben (Pro Technical CV. Mustika Lab), sodium metabisulfite (Pro Technical CV. Mustika Lab), and distilled water (Pro Technical CV. Mustika Lab), 96% ethanol (Pro Technical CV. Mustika Lab), propyleneglycol (Pro Technical CV. Mustika Lab), and hair tonic with Brand X Batch No. 187021.

Test animal

The animals used in this experiment were white male rabbits aged 3-4 months with a body weight of 2-3 kg. The number of rabbits was determined using the empirical formula of Federer, $(n\text{-}1)\ (t\text{-}1) \geq 15$, where t is the number of treatments and n is the number of animals per treatment (Juhaeni, 2014; Priskila, 2012). Based on this formula, the test animals included 4 white male rabbits.

Research Procedure

Screening of phytochemicals in the dry extract of *Aloe vera* and licorice

Flavonoid detection test

The simplisia powder was weighed as much as 0.5 g, added with 10 mL of methanol, refluxed for 10 minutes, and passed through a filter paper while the solution was still hot. The filtrate was diluted with 10 mL of distilled water. After it went cold, it was added with 5 mL of ether, shaken carefully, and left for a while. The methanol layer was removed and evaporated, while the rest was dissolved in 5 mL of ethyl acetate and filtered. The filtrate was used in the flavonoid detection test with the following procedure: A total of 1 mL of the solution was evaporated to dryness, while the

rest was dissolved in 2 mL of 95% ethanol and added with 0.1 g of magnesium powder and 10 drops of concentrated hydrochloric acid. A color change from orange or red to purplish red indicates the presence of flavonoids.

Terpenoid/steroid detection test

One gram of simplisia powder was extracted with 20 mL of ether for 2 hours and filtered. Each reagent was then examined using the following procedure: 3 drops of filtrate were added with 2 drops of Liebermann-Bouchard reagent. A change of color from purple or red to purplish blue or greenish blue indicates the presence of terpenoids/steroids.

Tannin detection test

Several samples were extracted with 20 mL of 70% ethanol. One milliliter of the resultant solution was added with 2 drops of 1% $FeCl_3$ solution. A positive reaction to tannins is shown by the emergence of blackish blue or blackish green color.

Saponin detection test

The simplisia powder was weighed as much as 0.5 g and put into a test tube. It was added with 10 mL of hot water, cooled, and then shaken vigorously for 10 seconds. The presence of saponin is indicated by stable foam with a height of 1-10 cm that is formed in less than 10 minutes and does not disappear after the addition of 1 drop of 2N hydrochloric acid.

The preparation of the concentration of *Aloe vera* and licorice extract

In 1 kg of the dry extract of *Aloe vera*, there were 80% of *Aloe vera* and 20% of maltodextrin. A concentration of 7.5% was created by adding 9.375 grams of *Aloe vera* extract with distilled water up to 100 mL One kilogram of the dry extract of licorice contained 75% licorice and 25% maltodextrin. The concentration of 2.5% was made of 3.3333 grams of licorice extract added with distilled water up to 100 mL.

Hair tonic preparation

The hair tonic was formulated using 7.5% of *Aloe vera* extract and 2.5% licorice extract as active ingredients, as well as varying percentages of 96% ethanol, namely 5%, 10%, and 15% (Table I). This dosage form was also evaluated based on its organoleptic properties, pH, specific weight, viscosity, and hedonic test results.

Table I. Hair tonic formula with a combination of *Aloe vera* L. and *Glycyrrhiza glabra* L. (licorice) extracts

Ingredients	Amount (%)		
	F1	F2	F3
Aloe vera extract	7.50	7.50	7.50
Licorice extract	2.50	2.50	2.50
96% Ethanol	5.00	10.00	15.00
Menthol	0.10	0.10	0.10
Sodium metabisulfite	0.10	0.10	0.10
Propylenglycol	10.00	10.00	10.00
Methylparaben	0.20	0.20	0.20
Distilled water (up to)	100.00	100.00	100.00

Methylparaben was dissolved in propylene glycol, while menthol was dissolved in ethanol. These two solutions were mixed, forming Solution 2. Sodium metabisulfite was dissolved in distilled water, stirred until homogeneous, and then added to the mixture of extracts that had been previously dissolved in distilled water. Solution 2 was combined with the extract mixture. The remaining distilled water was added a little at a time and stirred until homogeneous.

Hair tonic evaluation

The organoleptic test included visual observation on color, smell, and homogeneity. pH was measured with pH meter that had been calibrated by dipping the electrode in two solutions under the assumption that the pH of the test solution was between the pH of the two solutions. The commonly used solutions are pH 4 and pH 7. The specific weight was measured with wet and dry pycnometer. At room temperature, the empty pycnometer (w_1) was weighed and filled with distilled water, while the outside of the pycnometer was wiped dry and weighed (w_2). The distilled water was discharged. The pycnometer was dried and, then, filled with the hair tonic and weighed (w_3). The viscosity was identified using the Ostwald viscometer by measuring the time that the liquid required to pass through two marks as it flowed through a vertical capillary tube. The hedonic test was performed on the scent, sensation on the scalp, and color. The 5-point hedonic scale was (0) dislike, (1) neutral, (2) like slightly, (3) like, (4) like very much, and (5) like extremely. The panel consisted of 30 somewhat trained panelists.

Preparation of test animals

The rabbits went through an adaptation process for a week. Their back was divided into six areas of treatment with a width of 2.5x2.5 cm^2 and 1-cm distance between the areas. The next step was shaving the rabbit's hair in these six areas, followed by applying 70% ethanol as antiseptic.

Test for hair fertilizer activity

The hair tonic activity was analyzed by measuring hair growth rate, hair growth acceleration, and the weight of the rabbit's hair. One milliliter of hair tonic was applied (dropped) twice a day (morning and afternoon) to each area of treatment. During the 28-day observation, six rabbit hairs were taken from every area on day 7, 14, 21, and 28. The first day of hair tonic application was defined as day 0.

The hairs were cut, straightened, and taped on a dark colored base. They were measured with a caliper. On Day 28, all of the hairs in each area of treatment were cut and weighed. The six treatments were formula 1, formula 2, formula 3, positive control, negative control, and normal control. The positive control was a mixture of 7.5% of *Aloe vera* extract and 2.5% of licorice extract, the negative control was distilled water, and the normal control represented no treatment. This research used random positions for the treatments in each rabbit (Purwantini, 2008).

Data Analysis

The hair length and weight were evaluated statistically using One-way ANOVA or Kruskall-Wallis test, followed by Mann-Whitney test with a significance level of 0.05.

RESULTS AND DISCUSSION

The aqueous extract of *Aloe vera* is positive for flavonoid content, as indicated by the emergence of orange to red color. The formation of foam also proves that this extract has saponin. Saponin is a compound consisting of hydrophilic and hydrophobic groups. Following agitation, a hydrophilic group binds with water, whereas a hydrophobic group binds with air and creates froth. The addition of 2N HCl was to increase polarity that the hydrophilic groups would bind more firmly and the froth would be more stable (Kumalasari, 2011). The aqueous extract also positively contains tannins due to the emergence of a blackish blue color after the addition of $FeCl_3$. This color change is

the result of $FeCl_3$ reacting to one of the hydroxyl groups in tannins. Furthermore, this extract consists of triterpenoid. The steroid/triterpenoid content was detected based on the ability of the compound to form a color with concentrated H_2SO_4 in an anhydrous acetic acid solvent (Sangi, 2008).

As indicated by the orange or red color, the licorice extract is also positive for flavonoids. The emergence of stable foam, purplish-blue color, and blackish-blue color represents saponin, triterpenoid, and tannin content, respectively.

Hair tonic evaluation results

All of the three hair tonic formulas showed a brown color with typical odor of menthol. The resultant hair tonic dosage form was not transparent because the extract was dark brown. Specific weight is the ratio of the weight of a substance to the weight of water with the same volume, all of which are weighed in the air and at the same temperature. Measured with a pycnometer, the specific weight of formula 1, formula 2, and formula 3 were 1.0650 g/mL, 1.0600 g/mL, and 1.0577 g/mL. A heavier specific weight is induced by a higher distilled water content. The specific weight of the hair tonic was in line with this positive relationship. Among the three formulas, formula 1 had the highest amount of distilled water and, therefore, the highest specific weight.

Hair tonic is a dilute solution, which constitutes a Newtonian fluid. Therefore, the viscosity was quantified with a tool for measuring Newton flow, namely the Ostwald viscometer. The viscosity of a Newtonian fluid is directly proportional to the force and the shear rate. The higher the viscosity of a dosage form, the larger the force per unit area required to produce a shear rate (Martin, 1993). The flow time of the hair tonic was compared to the flow time of distilled water. The flow time (*t*) of the hair tonic created with formula 1 was longer than the one produced with formula 2 and 3. Since flow time is proportional to viscosity, formula 1 thereby provides hair tonic with the highest viscosity. The viscosities of formula 1, 2, and 3 were respectively 4.5983, 3.6458, and 2.8479 cP to distilled water. The higher the viscosity, the thicker the hair tonic. Viscosity is directly proportional to the molecular weight of the solute. The presence of heavy solute inhibits or gives a heavy load on the liquid, which increases viscosity (Kartika, 1990).

Formula 1, 2, and 3 did not have significantly different pH. Their pH values were 5.4, 5.2, and 5.5, respectively. These figures are still within the range of skin's pH (4.5-6.5); therefore, they do not cause irritation or itchy scalp. The hedonic or preference test relied on three parameters of preference, namely scent, color, and sensation on the scalp, from 30 untrained panelists. The results were as follows. (1) The smell of formula 2 was most preferred by the panelist because it used a fair amount of 96% ethanol. (2) The most preferred color was produced by formula 1. (3) Formula 3 was most preferred because it contained the most substantial amount of ethanol that introduced a cold sensation.

The Fertilizing Activity of Hair Tonic Dosage Form

The growth rate of rabbit's hair with hair tonic treatment

The hair length continually increased from Day 0 to Day 28. As seen in Table II, such increase already started even on Day 7. The hair treated with all of the three formulas was longer than all controls. Therefore, all of the hair tonic formulas in this research were able to increase hair growth activity better than the controls. Formula 3 produced the best hair length. There was no significant difference between the hair growth rates in normal and negative control. The base of the dosage form (normal control) exhibited no better hair growth activity in the untreated group.

On Day 28, the statistical tests showed that the data was distributed normally and homogeneously, which guaranteed its further analysis with ANOVA. There was a significant difference in hair length in each treatment group ($p < 0.05$). The Mann-Whitney test results showed that the normal control did not differ significantly to the negative control ($p > 0.05$). Therefore, the base of the hair tonic had the

same hair growth activity as the normal control. All of the three hair tonic formulas grew significantly different hair length from the negative, normal, and positive controls ($p < 0.05$).

Tabel II. The growth rate of rabbit's hair treated with tonics made of the aqueous extract of *Aloe vera* L. and *Glycyrrhiza glabra* L. (licorice) from Day 0 to Day 28

Day / Treatment	Hair Length (cm) ± SD			
	7	14	21	28
Formula 1	0.9763 ± 0.1107	1.2217 ± 0.1389	1.5983 ± 0.1617	2.230 ± 0.1365
Formula 2	1.0942 ± 0.0189	1.3321 ± 0.1541	1.8433 ± 0.0267	2.5333 ± 0.1684
Formula 3	1.1446 ± 0.1124	1.3479 ± 0.1442	1.8396 ± 0.0560	2.6617 ± 0.1369
Normal control	0.7596 ± 0.0968	0.9821 ± 0.1655	1.0238 ± 0.1219	1.3125 ± 0.1251
Positive control	0.9333 ± 0.1216	1.2829 ± 0.1835	1.3904 ± 0.0686	1.8596 ± 0.0495
Negative control	0.7208 ± 0.1186	0.9863 ± 0.1789	1.0367 ± 0.1436	1.3283 ± 0.1275

The comparison of hair growth accelerations using hair tonics and positive control

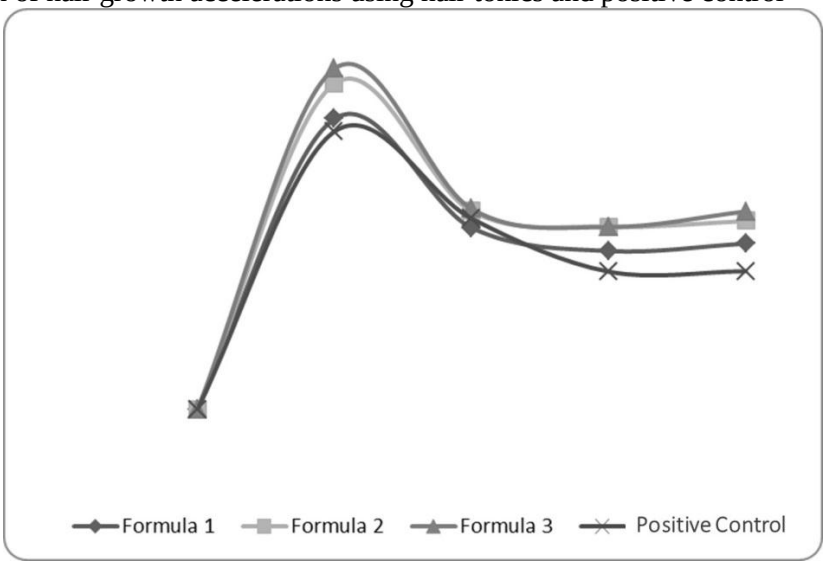

Figure 1. A graphic of hair growth acceleration from the application of hair tonic with a combination of the aqueous extract of *Aloe vera* L. and *Glycyrrhiza glabra* L. (licorice) from day 0 to day 28 compared to the positive control

Based on Figure 1, the hair growth rate induced by all hair tonics and the normal control increased from Day 0 to Day 7 with the highest growth acceleration in formula 3, followed by formula 2 and formula 1. However, from Day 7 to Day 21, there was a decrease in the hair growth rates of all hair tonic formulas and the normal control. In the next seven days (Day 21-28), the rate from all hair tonics slightly increased, but there was no increase in the normal control. The normal control continued to decrease the hair growth rate until Day 28. Formula 3 and 2 showed a slightly different decrease in the growth rate. The fastest growth rate occurred on Day 7 due to the treatment

with formula 3, i.e., 0.1635 cm/day. All of the hair tonic formulas exhibited an activity of increasing the hair growth rate better than the untreated (normal) group.

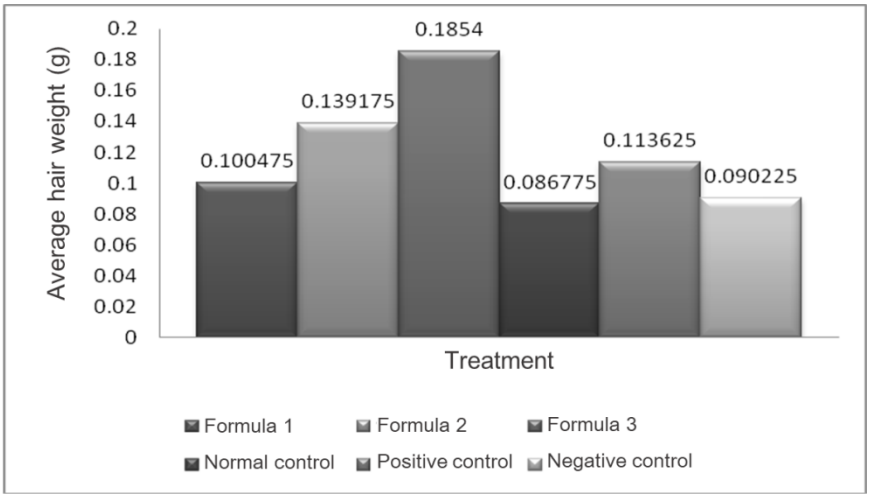

Figure 2. The chart of the weight of rabbit's hair after the application of hair tonic with a combination of the aqueous extract of *Aloe vera* L. and *Glycyrrhiza glabra* L. (licorice) on day 28

Based on Figure 2, the rabbit's hair after the treatment with the hair tonics was heavier than in the normal control and negative control. In other words, the hair tonic exhibited an activity of increasing hair weight. Formula 3 produced the heaviest hair, followed by formula 2 and 1.

The statistical test results showed that the average hair weight was normally but not homogeneously distributed. Therefore, this data was further analyzed with the Kruskal-Wallis test. The results showed that there was a significant difference of hair weight in each treatment group. The Mann-Whitney test proved that the normal control and the negative control showed no significant difference ($p > 0.05$) or, in other words, both controls exhibited the same hair growth activity. The hair weight treated with formula 3 showed a significant difference from the negative control and the normal control ($p < 0.05$). Formula 3 had better hair growth activity than the three controls.

The proteolytic enzyme in *Aloe vera* exfoliates dead skin cells and opens the pores of the cells, as well as improves membrane fluidity and permeability, toxin outflow, and nutrient flow (Reynolds and Dweck, 1999; Moon *et al*., 1999). *Aloe vera* can even repair vascular tissue (angiogenesis) (Heggars *et al.*, 1993). It contains lignin and polysaccharide as nutrient carrier required by the skin with normal pH. Its pH is similar to human skin's; therefore, the nutrient can penetrate into the hair tissue. *Aloe vera* has 20 types of amino acids that help the development of new cells and remove dead cells from the epidermis (Bassetti, 2005). These amino acids can moisturize epidermis, soften hair, increase the size of hair follicles, and function as antibacterial (Dutt, 2002).

Isoflavones, glycyrrhizin, and glycolytic acids in licorice have estrogen-like activity and can decrease testosterone levels (Grant, 2012). Estrogens can modify androgen metabolism in different hair follicle subunits, thereby reducing the 5α-dihydrotestosterone content and influencing the growth factor and the transcription of cytokines, i.e., hormones that play a role in normal hair growth (Ohnemus, 2006).

Increased penetration of ethanol in hair growers (minoxidil as the active ingredient) plays an essential role in optimum treatment. A formulation with 50% of 96% ethanol nearly doubles the penetration. The penetration increases by three folds when 75% of ethanol was used and up to eight times with 90% of ethanol. The volatile nature of ethanol increases the concentration of the extract on the skin (increased thermodynamic activity likely carries the active substance through the stratum

corneum) or improves the ability of active substances to penetrate the skin because ethanol changes the physical integrity of the stratum corneum barrier (Tata *et al*., 1995).

CONCLUSION

Based on the results concluded that hair tonic with formula 3 showed a significant difference from the negative control. Formula 3 resulted in a hair growth rate of 2.6617 cm, hair growth acceleration of 0.1635 cm/day, and hair weight of 0.1854 gram in 28 days. Hair tonic with this formula was stable during the 30-day storage. Its characteristics included brown color with a typical odor of menthol, a specific weight of 1.0577 g/mL, a viscosity of 2.8479 cP to distilled water, and pH 5.5.

REFERENCES

Bassetti, A., Sale, S. 2005. *The Great Aloe Book*. USA: First edition, Zuccari edition.p 77

Anonymous, 1985, *Formularium Kosmetik Indonesia*. Direktorat Jendral POM Jakarta: Departemen Kesehatan RI ; p 86.

Dutt, B., 2002, A study of Patenting Activity in *Aloe vera* , *Jurnal of Intelectual Property Rights*.7 : 330-341.

Grant, P., Ramasamy, S., 2012. An Update on Plant Derived Anti-Androgens, *International jurnal of Endocrinology Metabolism*.10(2):497-502.

Heggars, J.P., Pelley, R.P. and Robson, M.C., 1993, Beneficial effects of aloe in wound healing. *Phytother. Res*., 7: S48-S52.

Jain, R., Neetesh, K.J., Namrata, S., Gnanachandran, A.K., Gokulan, P.D., 2011. Development And Evaluation Of Polyherbal Ointment For Hair Growth Activity, *International Journal of Pharmacy and Pharmaceutical Sciences*; 3(2):180-182.

Juheini, A., Esther L.P.S., Anwar, E., Djajadisastra, J., 2014. Green Tea (Camellia sinensis, L.) Ethanolic Extract as Hair Tonic in Nutraceutical:Physical Stability, Hair Growth Activity on Rats, and Safety Test, *International Journal of Pharmacy and Pharmaceutical Sciences*. 6 (5).94-99.

Kartika. 1990. Viskositas.Makassar : Universitas Hasanuddin.

Kumalasari, E. and Sulistyani, N., 2011. Aktivitas antifungi ekstrak etanol batang binahong (*Anredera cordifolia* (Tenore) Steen.) terhadap *Candida albicans* serta Skrining Fitokimia, *Jurnal Ilmiah Kefarmasian*; 1(2): 51 – 62.

Martin, A., Swarbick, J., Cammarata, A., 1993. *Farmasi Fisik*. Universitas Indonesia.

Mitsui, T., Ed.1996. *New Cosmetic Science*, Part II, Hair Cosmetics. Elsevier. Amsterdam; 413.

Moon, E.J., Y.M. Lee, O.H. Lee, M.J. Lee and S.K. Lee, M.H. Chung, Y.I. Park, C.K. Sung, J.S. Choi, K.W.Kim, 1999. A novel angiogenic factor derived from Aloe vera gel: Beta-sitosterol, a plant sterol. *Angiogenesis*, 3: 117-123.

Ohnemus, U., and Uenalan M., 2006. The hair follicle as an estrogen target and source, *The Endrocrine Society*. 27(6): 677-706.

Priskila, V., 2012. Uji stabilitas fisik dan uji aktivitas pertumbuhan rambut tikus putih jantan dari sediaan hair tonik yang mengandung ekstrak air bonggol pisang kepok (*musa b albisiana*) *Skripsi*. Depok : Fakultas MIPA. Program studi farmasi.

Purwantini, I., Rima, M., Naniek, D.B.S., 2008. Combination of teh and mangkokan leaves extract to promote hair growth, *Traditional Medicine Journal*. 13(43).

Reynolds, T. and A.C. Dweck., 1999, Aloe vera leaf gel: A review update. *J. Ethnopharmacol*, 68: 3-37.

Rostita. 2008. Sehat, Cantik dan Penuh Vitalitas Berkat Lidah Buaya. Bandung: PT Mizan Pustaka.

Sangi, M., M.R.J. Runtuwene., H.E.I. Simbala, and V.M.A. 2008. Makang. Analisis Fitokimia Tumbuhan Obat di kabupaten Minahasa Utara. *Chem. Prog*; 1(1):47-53.

Sukirti, U., Ashoke KG, Vijender, S., 2012. Hair growth promotant activity of petroleum ether root extract of *Glycyrrhiza Glabra* L (Fabaceae) in female rats, *Tropical Journal of Pharmaceutical Research*; 11(5):753-758.

Tata, S., Flynn, G. L., and Weiner, N. D., 1995. Penetration of minoxidil from ethanol/propylene glycol solutions: effect of application volume and occlusion, *Journal of pharmaceutical sciences*; 84(6), 688–91.