

1.0 INTRODUCTION

Biophysical skin parameters are indicators of age related structural and functional changes in skin tissues. They are usually used to describe the efficiency of skin care products either it is taken orally or applied topically. In this study, 6 healthy adult volunteers were used to evaluate the effects of Living Jungle PP Mask on biophysical skin parameters. The biophysical parameters will include skin moisture, firmness, elasticity, transepidermal water loss (TEWL), roughness, smoothness and wrinkle.

Living Jungle PP Mask is formulated with 7 key ingredients containing skin nutrients that can penetrate deep inside the skin resulting in brighter and gorgeous skin. Synergistic effects of these 7 key ingredients in Living Jungle PP Mask resulted in instantly fairer and fresh skin. It helps to remove blackheads, whiteheads and improve skin elasticity. Daily usage will also reduce uneven pigmentation effects. Hence, we predicted that Living Jungle PP Mask will restore a defective epidermal skin barrier, normalize excessive transepidermal water loss (TEWL) and improve the smoothness of the skin after application.

2.0 OBJECTIVES

To determine the effects of individual topically applied Living Jungle PP Mask on the biophysical skin parameters

3.0 MATERIALS AND METHODS

3.1 Subjects

6 healthy males and females between 25-69 years of age participated as volunteers for this study. Prior to the tests, every volunteer was provided with a volunteer protocol. This protocol stated the terms and conditions of the testing. Every volunteer was required to read, understand and agree to the terms and conditions stated. Volunteers were informed on the contents of the formulations. The subjects were briefed on the product usage and instruction. Measurement was taken before and after product usage. Before the measurements were taken, the subjects were acclimatized for 20 minutes to a room temperature of 25°C.

3.2 Materials

Living Jungle PP Mask is enriched with 7 key ingredients offering great benefits for beautiful radiant skin. Licorice as natural whitening agent, apple stem cells provides rejuvenation effects, orange stem cells supply antioxidant and vitamin C, rice bran is well known as great ancient's moisturizer, aloe vera gives antibacterial effect, green tea also provides natural antioxidant and lastly diamond helps in energizing our skin by improving aura.

3.3 Measurements

The following skin parameters were measured: roughness, smoothness, wrinkle, moisture, TEWL, firmness, and elasticity. All biophysical parameters were measured three times on the forehead and left cheek of the subjects (Figure 1) and results were averaged.

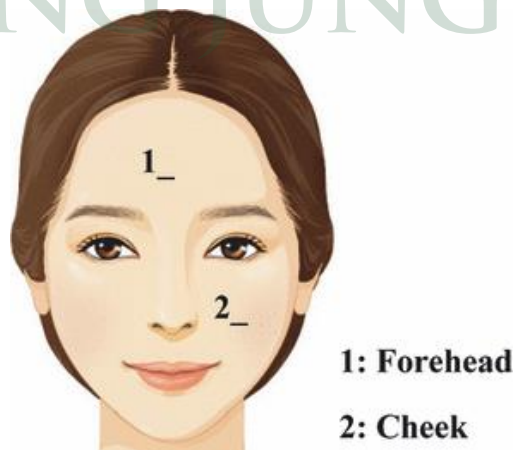


Figure 1: The biophysical parameters were measured on the face (forehead and cheek).

3.3.1 Non-invasive in vivo skin tests

3.3.1.1 Skin topography

Skin topography is obtained through shadowing casting method of surface image analysis by using Skin Visioscan VC98 (Courage & Khazaka, Germany). It is used to visualize the micro relief and wrinkle of the featured skin surface. The Skin Visioscan VC98 is placed on the forehead and cheek front (left) (Figure 1) of each subject. Analysis of the Skin Visioscan VC98

includes Surface Evaluation of the Living Skin (SELS) parameters which involve skin roughness, smoothness and wrinkles.

3.3.1.2 Skin hydration (humidity)

The skin moisture content was measured with the corneometer CM 825 PC (Courage + Khazana, Germany) which measures the electrical capacitance of the skin surface. The capacitance is expressed digitally in arbitrary units. The measuring principle is based on the large difference of the dielectric constant of water and that of most other materials. The probe head (7 x 7 mm) with condenser was applied to the skin surface at a constant force. Subjects were examined in a closed room with a controlled temperature following acclimatization in the room for approximately 20 minutes.

3.3.1.3 Biomechanical skin properties

The biomechanical skin properties measurement includes elasticity, firmness and fatigue resistance according to method performed by Muggli R. (2005). These skin parameters were assessed by a non-invasive suction and elongation method of the Cutometer MPA580 (Courage & Khazaka, Germany). The measurement is based on the suction principle. By applying a defined under pressure, skin is drawn into a hollow tube with an orifice of 2mm in diameter. The skin is then allowed to retract at ambient pressure. The penetration depth of the skin into the tube is continuously recorded optically and frictionless and as a function of time. A number of standardized parameters can be calculated from the penetration depth curve. To increase the accuracy and to obtain information on the properties of skin under repeated external stress, the suction-release cycle is repeated several times. The study was conducted with 2 mm probe and a constant negative pressure of 450 mbar is applied for 4 s followed by a relaxation time of 2 s. Three area parameters were selected to assess the changes of three biomechanical skin properties:

(1) Skin firmness, defined by the area below the maximum envelope curve (F 4). A decrease in F4 corresponds to an increase in skin firmness.

(2) Skin elasticity, defined by the ratio $F3/F4$, where $F3$ is the area between the maximum and minimum envelope curve. An increase in $F3/F4$ corresponds to an increase in skin elasticity. The closer the ratio is to 1, the more elastic is the skin.



Figure 2: Biophysical skin testing by Tewameter (A) and Cutometer (B)

3.3.1.4 TEWL

Transepidermal water loss (TEWL) is a measurement of water quantity that passes from inside a body through the epidermal layer (skin) to the surrounding atmosphere via diffusion and evaporation process. It is measured to characterize the water barrier function of the skin. TEWL is measured by tewameter TM300 (Courage & Khazaka, Germany). The TEWL is calculated automatically and expressed digitally in $\text{g m}^{-2} \text{h}^{-1}$. The probe was held in impermeable gloves to avoid influence on the analysis. Continuous measurement was made on each site until standard deviation (SD) is lower than 0.01. Each value was the average of three different measurements on adjacent skin sites.

3.4 STATISTICAL ANALYSIS

The results of all parameters for placebo and treatment groups will be presented through mean \pm standard error of mean. Since the data are normally distributed and based on the fact that there will be more than two means to be compared, datasets for both groups was analyzed using parametric test which is one-way ANOVA test. The hypotheses formation of ANOVA test was used throughout the analysis.

Note:

$H0A : \mu 1A = \mu 2A = \mu 3A$

$H1A : \text{At least one mean is different from the others}$

$H0B : \mu 1B = \mu 2B = \mu 3B$

$H1B : \text{At least one mean is different from the others}$

After the formation of the hypotheses, the analysis will be carried out using SPSS version 16.0.

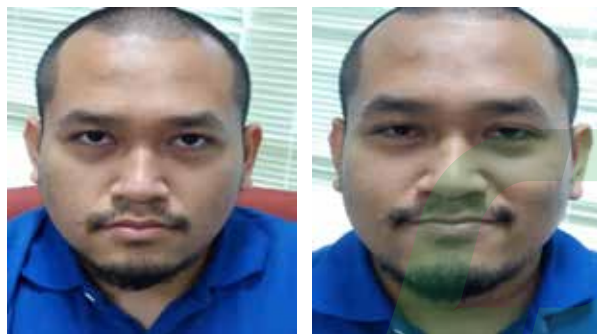
The significance of the results will be determined using post-hoc Duncan test[®]

4.0 RESULTS

4.1 Skin topography

Skin topography for 6 females and males between 25-69 years of age were analysed by the distinction of surface image analysis before and after apply Living Jungle PP Mask. All results obtained indicate positive effect after applying Jungle PP Mask. A marked reduction of skin roughness, wrinkles and dryness were clearly visible on the forehead and cheek area as shown in figure 3 (a)-(f). Additionally, a significant reduction in skin pores were also observed especially for female aged 40s as shown in figure 4 (a)-(b).

a) Male aged 20s



Before

After



Forehead before



Forehead after



Cheekbone before



Cheekbone after

b) Female aged 20s



Before

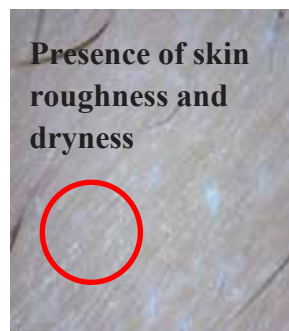
After



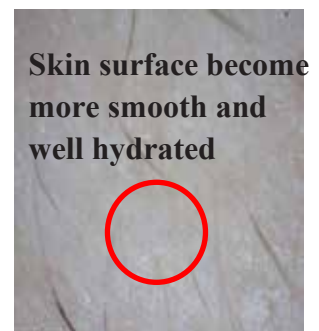
Forehead before



Forehead after



Cheekbone before



Cheekbone after

c) Male aged 40s



Before



After



Forehead before



Forehead after



Cheekbone before



Cheekbone after

e) Male aged 60s



Before



After

d) Female aged 40s



Before



After



Forehead before



Forehead after



Cheekbone before



Cheekbone after

f) Female aged 60s



Before



After

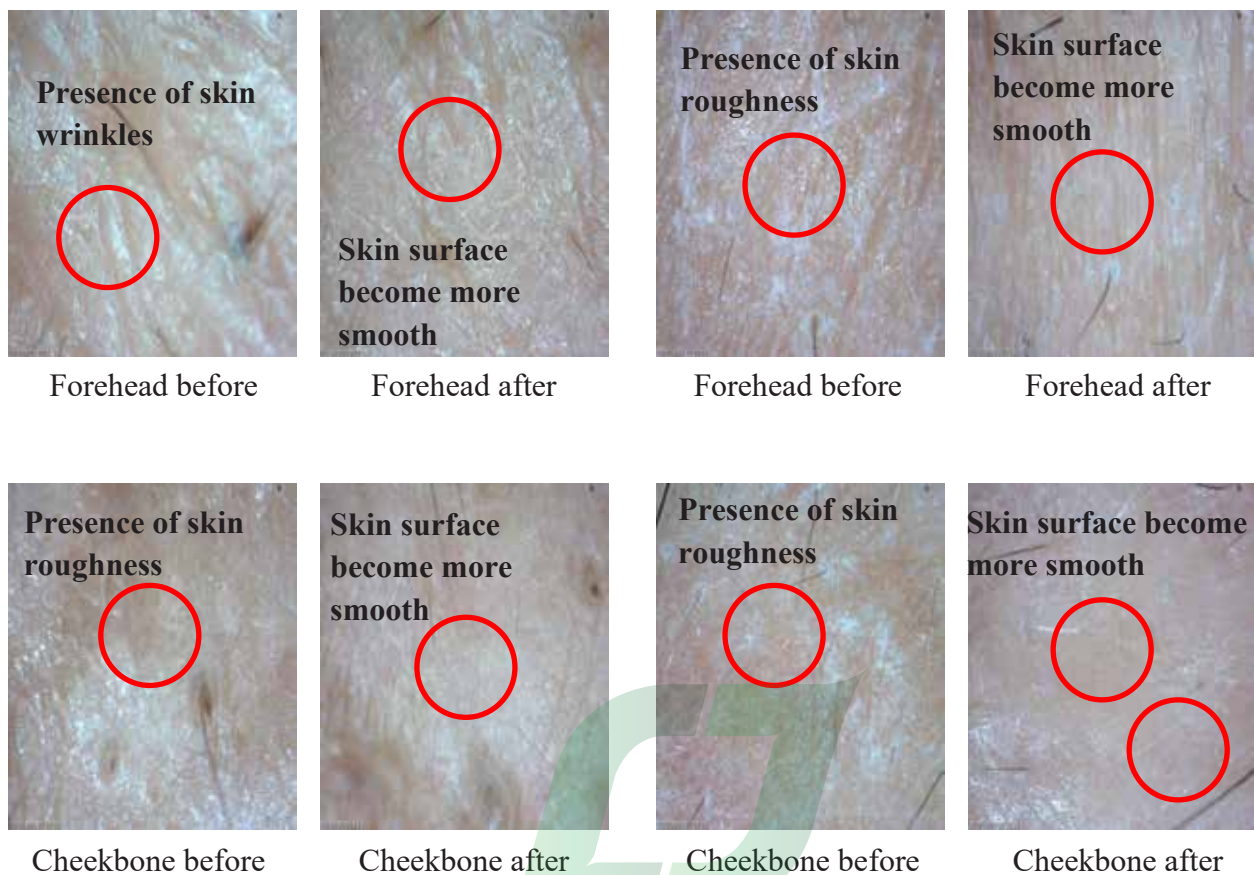


Figure 3: Comparison of skin topography a) Male age 20s, b) Female aged 20s, c) Male 40s, d) Female aged 40s, e) Male aged 60s and f) Female aged 60s before and after apply Living Jungle PP Mask on forehead and cheekbone area at different age and gender.

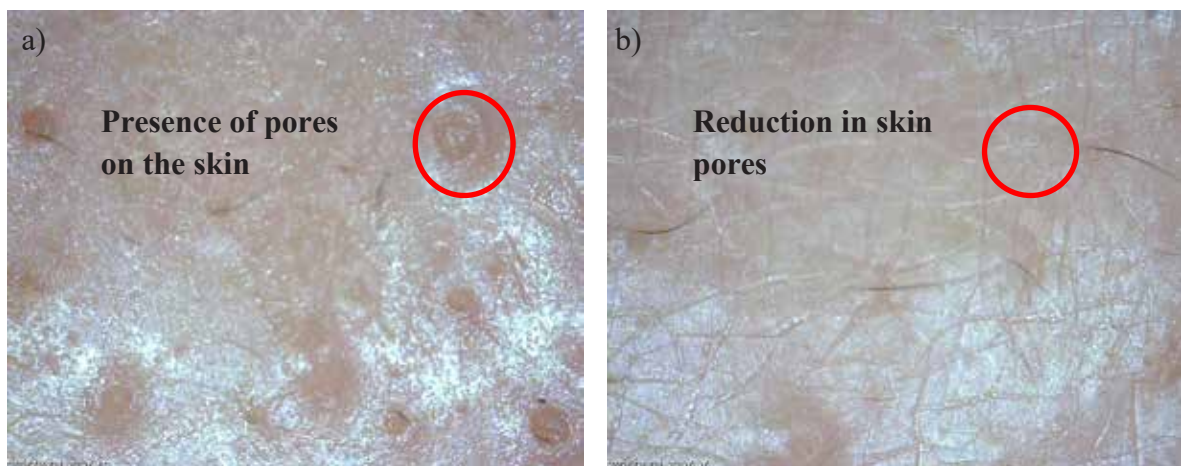


Figure 4: Comparison of skin topography a) before and b) after the application of Living Jungle PP Mask for female aged 40s.

4.2 Skin hydration

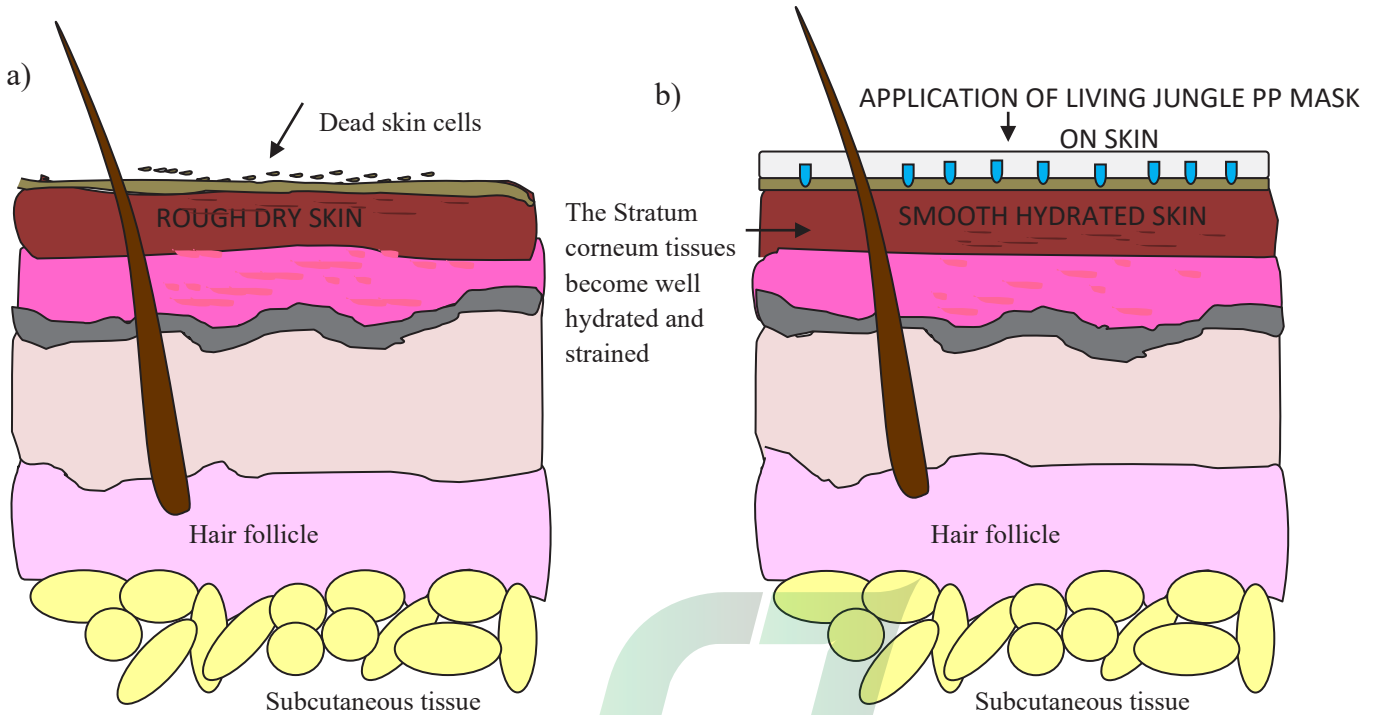


Figure 5: Illustration of a) less skin hydration and b) well hydrated and smooth texture skin and when apply Living Jungle PP Mask.

Figure 5 illustrates the comparison of less hydrated rough skin with well hydrated and smooth skin after applying Living Jungle PP Mask. Marked changes observed in the topography test for forehead area were supported by the significant increase in skin hydration value (ANOVA, $p < 0.05$) of forehead area of all tested subjects obtained following 1 h application of Living Jungle PP Mask which indicate positive effect (Figure 6). Cheekbone skin hydration also shows significant increase in value except for female aged 40s shows no significant difference from 88.40 ± 4.72 to 88.16 ± 2.38 before and after the application of Living Jungle PP Mask respectively as shown in figure 7.

Based on the result, the increase in hydration effect of Living Jungle PP Mask were able to achieve 5% to 70% for forehead and 2% to 25% for cheekbone area for all tested subjects. These results prove the ability to retain the moisture in skin as indicated in the improvement in morphological changes shown in the skin topography images.

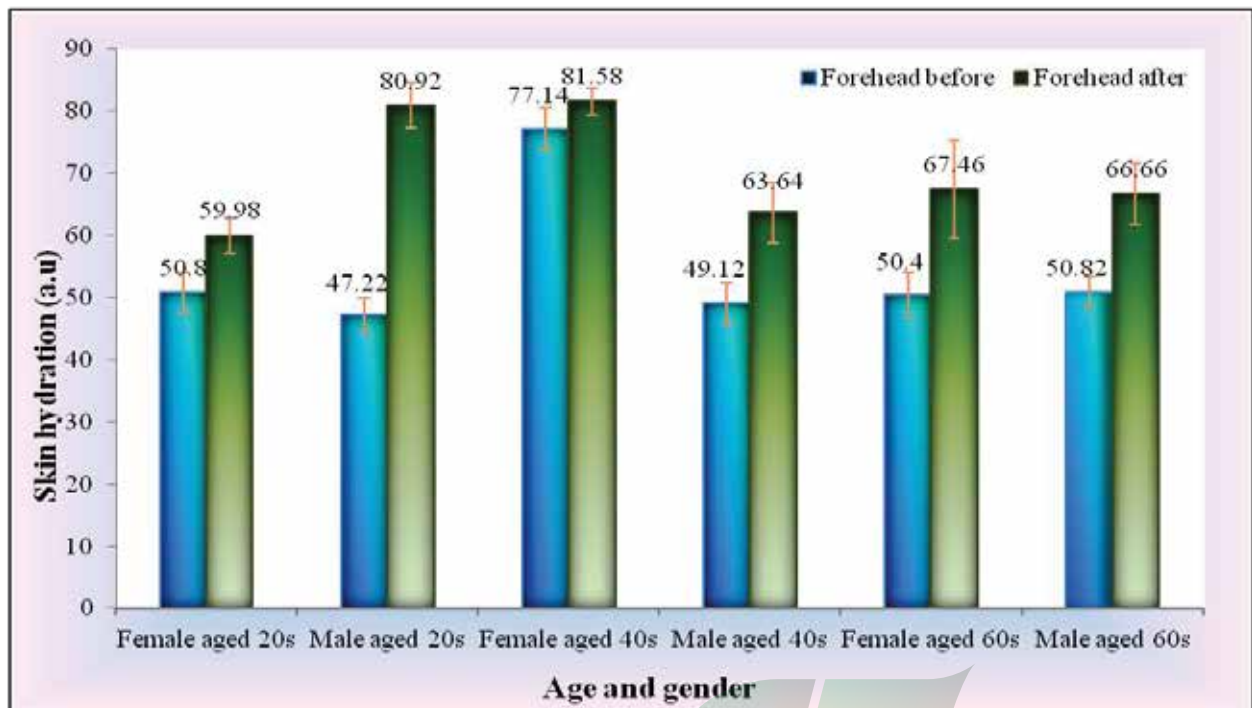


Figure 6: Comparison of the measured data for skin hydration on forehead area before and after using Living Jungle PP mask at different age and gender. The percentage increment of skin hydration stated from range 5% to 70% on forehead area for all tested subjects.

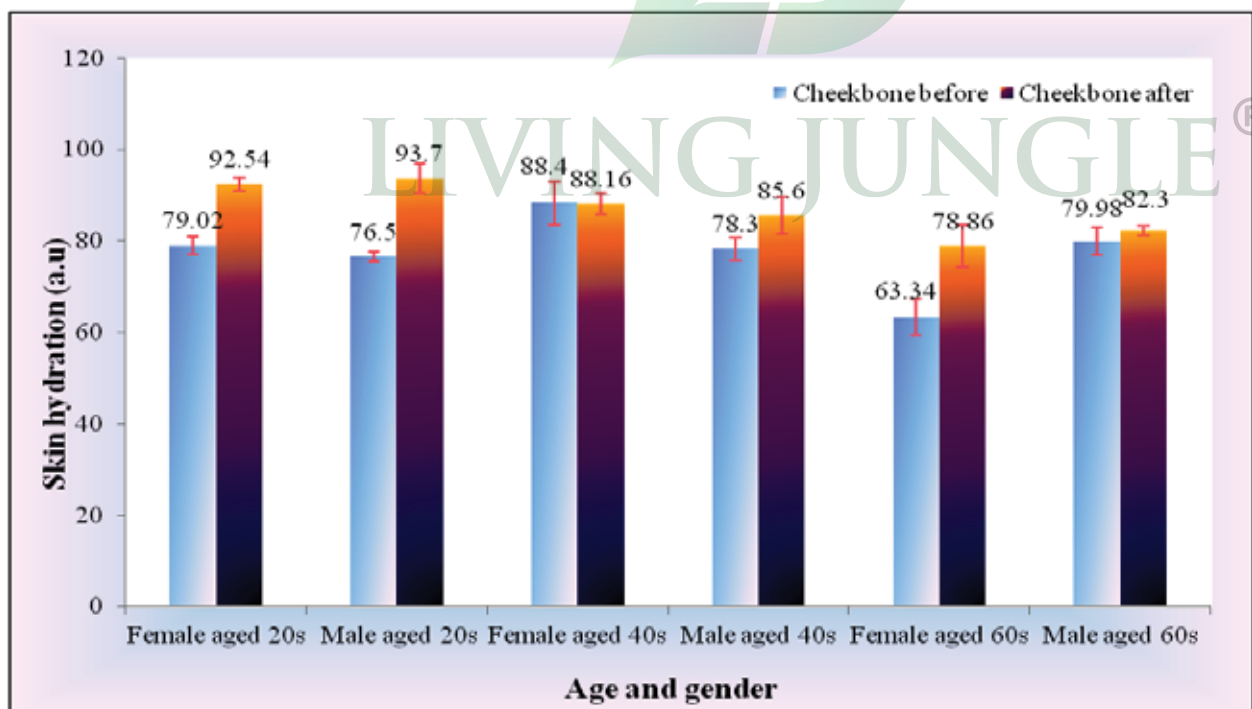


Figure 7: Comparison of the measured data for skin hydration on cheekbone area before and after using Living Jungle PP mask at different age and gender. The percentage increment of skin hydration stated from range 2% to 25% on cheekbone area for all tested subjects.

4.3 Melanin content

In line with positive results obtained from skin hydration and topography test, the melanin content were found to markedly decrease on the forehead and cheekbone area for all subject following the application of Living Jungle PP Mask for 1 h (Figure 8 and 9). The ANOVA test shows a significant decrease in melanin content ($P < 0.05$) in forehead and cheekbone after application of the mask except for female and male aged 60s. The melanin content indicates no significant difference before and after applying the mask from 262.67 ± 7.57 to 259.00 ± 3.61 and 370.33 ± 6.67 to 363.33 ± 2.31 for forehead and cheekbone area respectively (Figure 8 and 9; ANOVA, $p > 0.05$). Melanin production process (melanogenesis) involves melanocyte interaction with endocrine, immune, inflammatory and central nervous system which may deteriorates with aging factors thus causing regulation of it extrinsically may requires more time compared to young aged of 20s and 40s. It is able to describe that the percentage reduction of melanin content were range at 1% to 6% on forehead area whereas 1% to 31% on cheekbone area for all tested subjects as indication of a more radiant looking skin as compared to before the application of Living Jungle PP Mask.

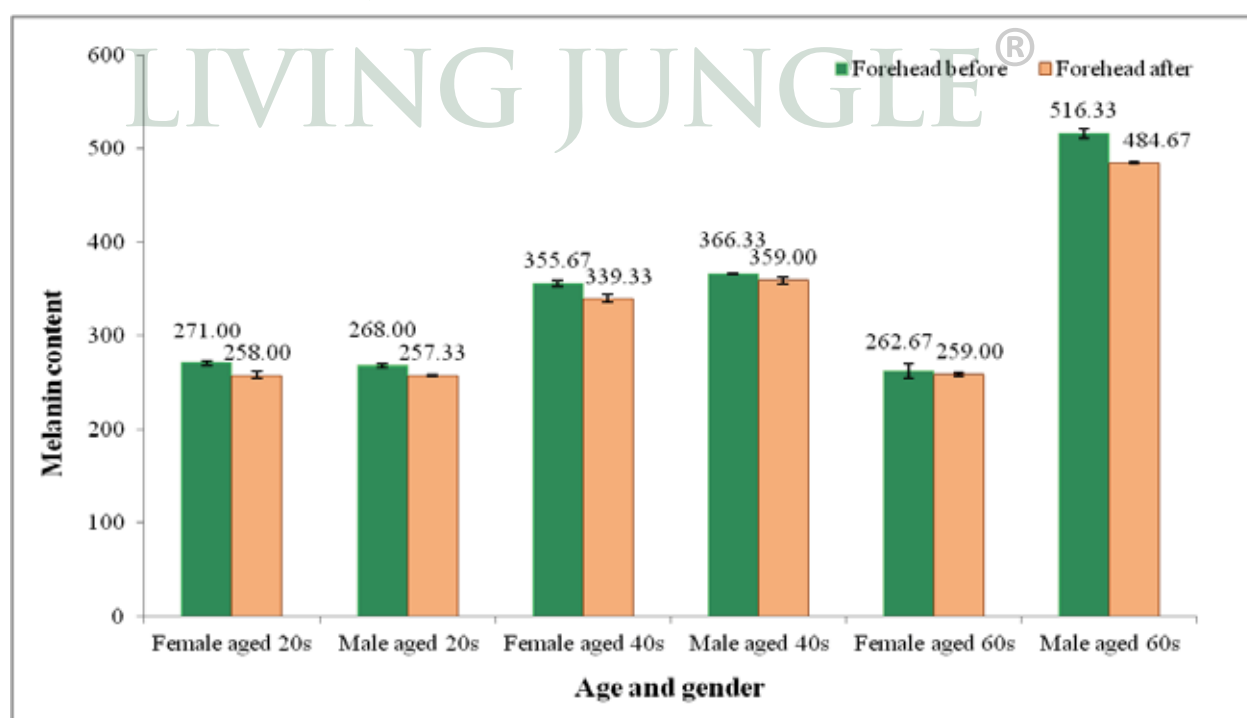


Figure 8: Comparison of the measured data for melanin content on forehead area before and after using Living Jungle PP mask at different age and gender. The percentage reduction skin melanin content stated from range 1% to 6% on forehead area for all tested subjects.

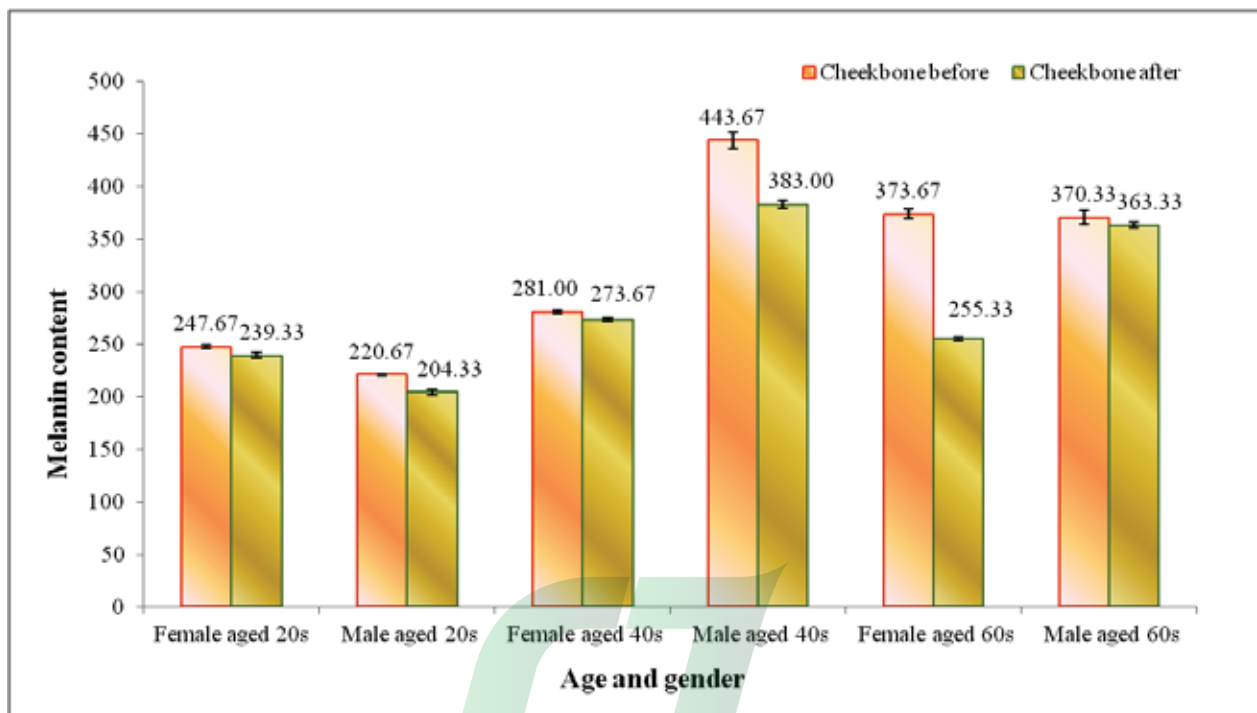


Figure 9: Comparison of the measured data for melanin content on cheekbone area before and after using Living Jungle PP mask at different age and gender. The percentage reduction of skin melanin content stated from range 1% to 31% on cheekbone area for all tested subjects.

4.4 Skin elasticity

Figure 10 and 11 exhibit the skin elasticity in forehead and cheekbone, before and after application Living Jungle PP Mask for 1 hour. The findings showed an increment in value of skin elasticity for all subjects except for male aged 20s and female aged 40s stated slight reduction in value from 0.408 ± 0.04 to 0.354 ± 0.03 and 0.459 ± 0.10 to 0.382 ± 0.03 respectively on forehead area. Meanwhile on cheekbone area only male aged 40s showed slightly reduction in skin elasticity value. Even though there are minimal reduction in value, some remarkable value of skin elasticity able to achieved up to 15% and 39% increment especially for the male aged 60s and female aged 40s on forehead and cheekbone area respectively. Normally, the skin elasticity able to apparent in high value within a few weeks of tested skin, however this mask were only applied for 1 hours then this may cause of minimal changes in value.

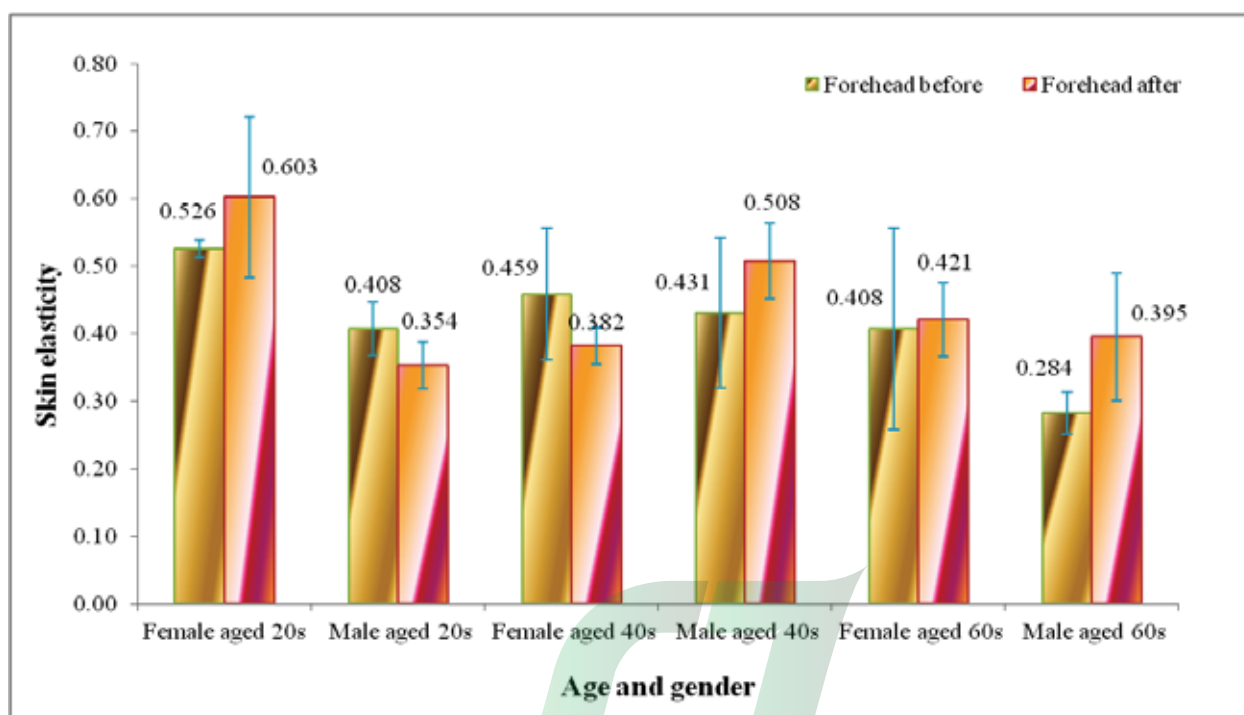


Figure 10: Comparison of the measured data for skin elasticity on forehead area before and after using Living Jungle PP mask at different age and gender. The percentage increments for the skin elasticity were achieved up to 39% on forehead area.



Figure 11: Comparison of the measured data for skin elasticity on cheekbone area before and after using Living Jungle PP mask at different age and gender. The percentage increments for the skin elasticity were achieved up to 15% on cheekbone area.

4.5 TEWL

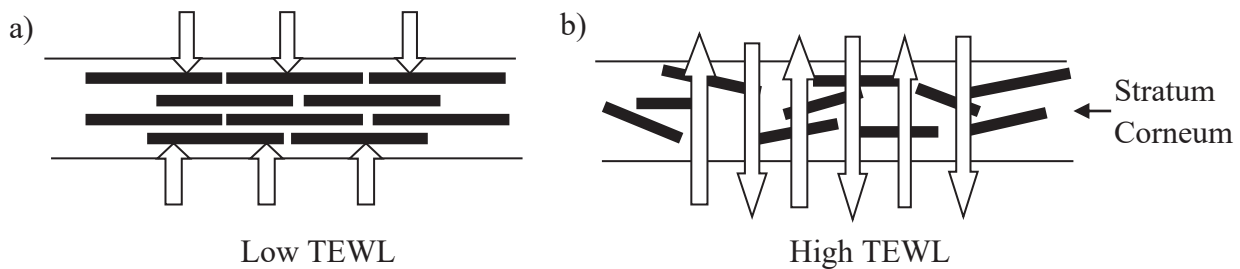


Figure 12: Schematic illustration of the barrier function of the stratum corneum of a) Healthy skin and b) Disturbed skin

In figure 12 a) and b) illustrated the comparison between the healthy skin and disrupted skin in the stratum corneum which is act as the main barrier function. Transepidermal water loss value indicates the effectiveness of skin barrier function corresponding to exogenic factors such as germs and bacteria respectively. In figure 13 illustrated the application of Living Jungle PP Mask on the skin in which the hypothesis is TEWL test value should be low as the skin should retain the moisture within it.

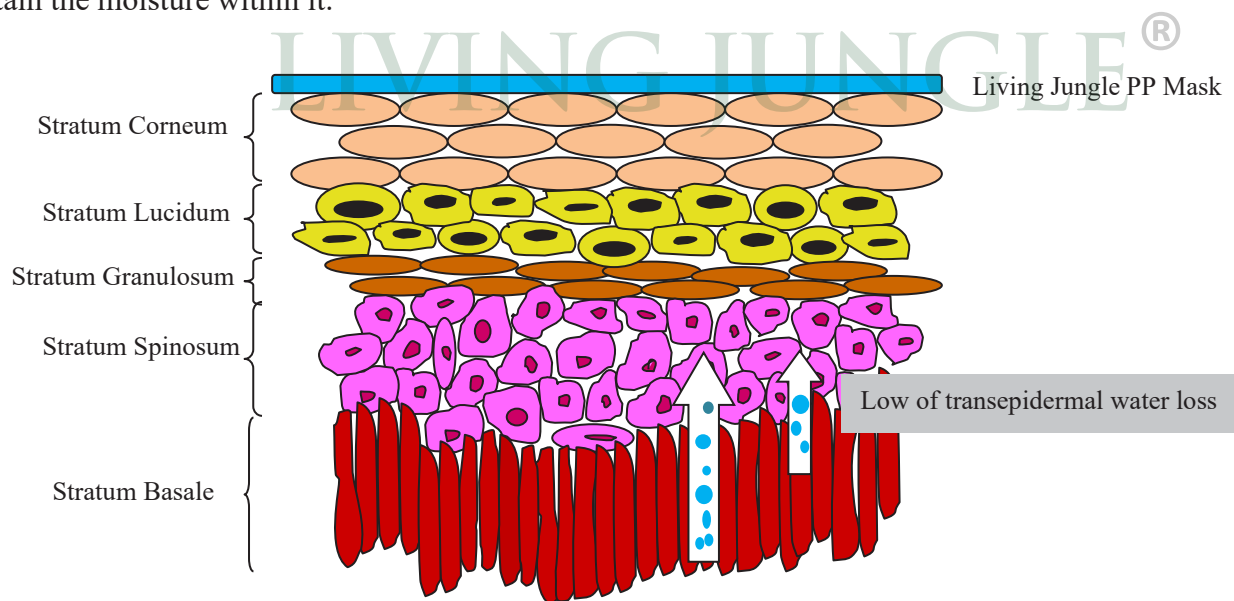


Figure 13: The illustration of application of Living Jungle PP Mask at outer layer of stratum corneum on the skin and demonstrate the TEWL mechanism.

Based on the graph in figure 14 and 15, the findings showed that transepidermal water loss in cheekbone area exhibits low value in the most of the subjects compared to forehead area after applying the Living Jungle PP Mask. This may be due to the variation in thickness at different area which may be reflected from regional variations in structure such as distribution of sweat eccrine glands, microvasculature and increasing size of corneocytes at outermost part of epidermis thus increases the epidermal permeability barrier of skin.

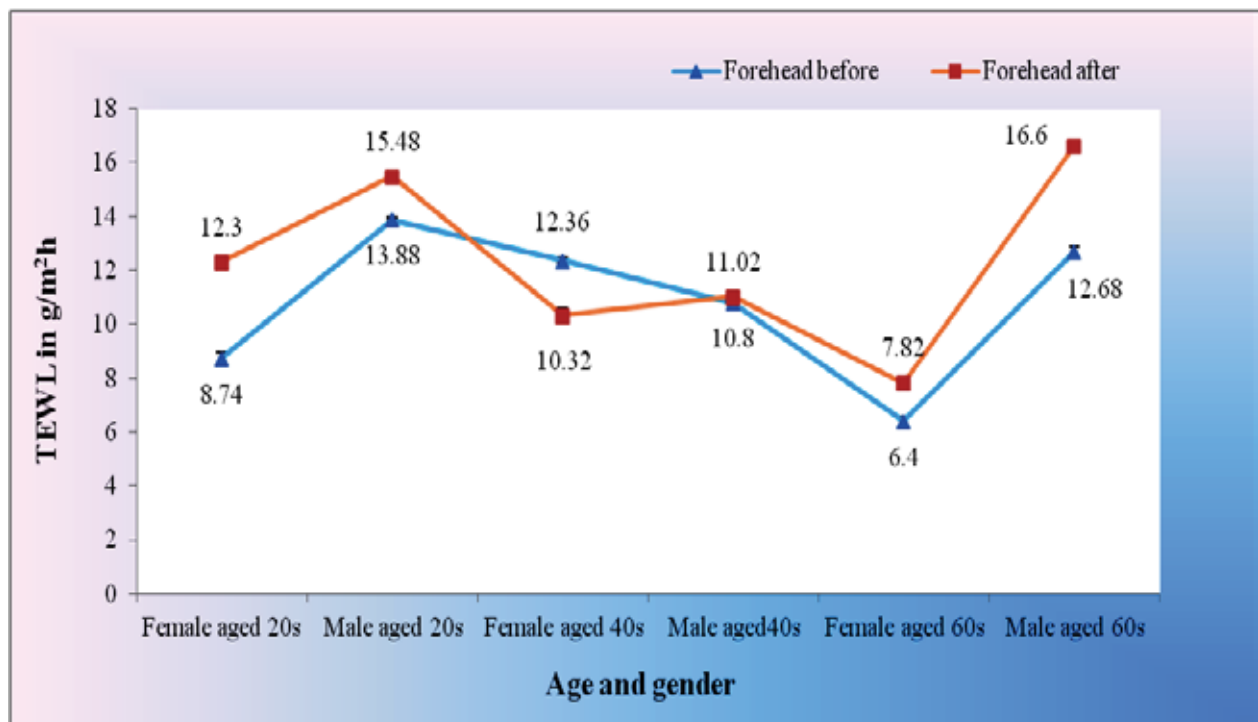


Figure 14: Comparison of the measured data for TEWL test on forehead area before and after using Living Jungle PP mask at different age and gender.

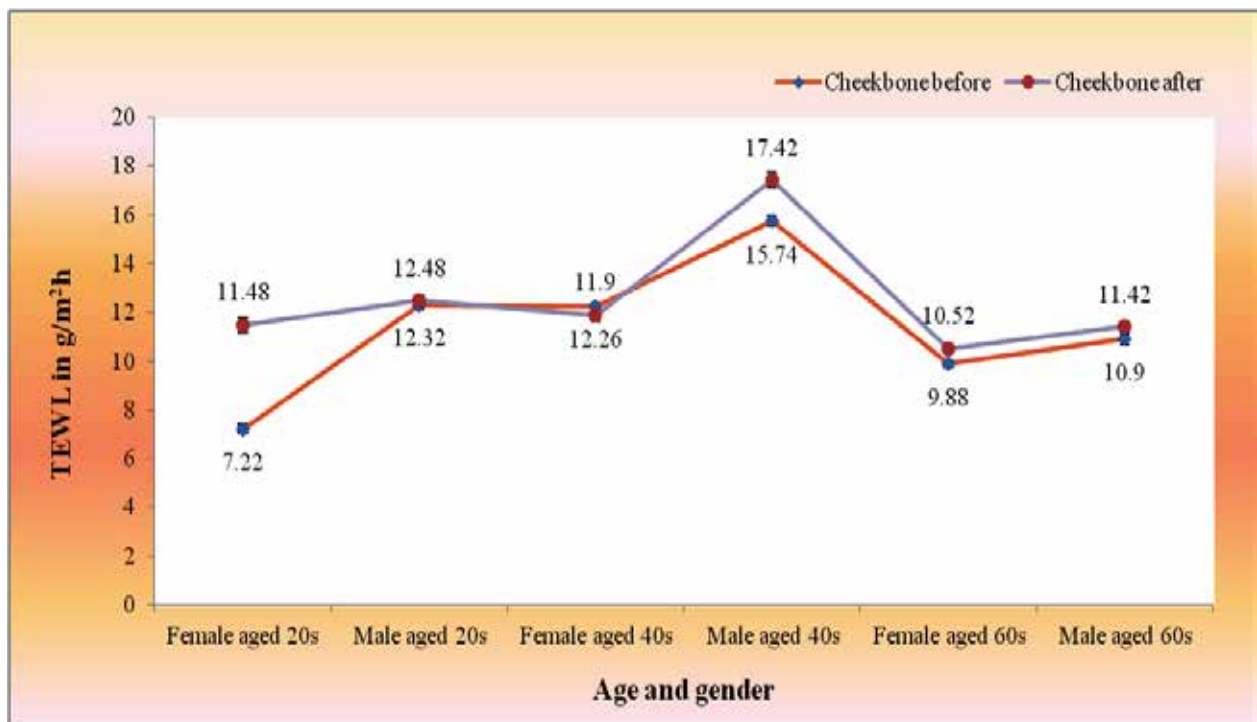


Figure 15: Comparison of the measured data for TEWL test on cheekbone area before and after using Living Jungle PP mask at different age and gender.

5.0 CONCLUSION

Living Jungle PP Mask containing 7 special key Ingredients including Licorice, Apple stem cells, Orange Stem Cells, Rice bran, Aleo Vera, Green Tea and Diamond represented as whitening agent, rejuvenates skin cells, antioxidant as well as vitamin C, moisturizer, antibacterial, natural antioxidant and energizing appearance respectively able to offer positive outcome through the non-invasive in vivo skin tests such as skin topography, skin hydration, biomechanical skin properties including elasticity, melanin content and TEWL in which showed a significant effective changes in the skin when applying Living Jungle PP Mask. These positive outcomes are clearly noticeable when the skin topography showed the reduction of skin roughness, wrinkles, and dryness as well as pores size on the skin. Parallel to this, the skin hydration showed the remarkable increment up to 25% and 70% on cheekbone and forehead area respectively. For the melanin content, reductions were observed in between 1% to 6% on forehead area and 1% to 31% on cheekbone area respectively. All subjects shows the radiant, fairness and fresh skin through this synergistic whitening and antioxidant effect of Living Jungle

PP Mask unique ingredients which protect cells against free radical and inhibit overproduction of melanin in the skin in order to prevent hyperpigmentation process. Even though some of the subject recorded a minimal reduction of skin elasticity value after Living Jungle PP Mask application, majority of the subjects shows an increased in skin elasticity values. A remarkable increased of skin elasticity ranging at 15% and 39% was observed in case of female aged 40s and male aged 60s on cheekbone and forehead area. In case of TEWL, there were a significant change on the skin moisture retain ability in most of the subjects tested even though PP Mask was used once. We believed that, improvements could be achieved through frequent and consistent used of Living Jungle PP Mask.

6.0 REFERENCES

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