Clinical Efficacy and Safety of an Anti-Cellulite Cosmetics containing high Content of Caffeine Complex and A Transdermal Penetrating Peptide

Young Il Kwon,Hoon Cha, Seon Hwa Kim, Su In Park, Moon Sam Shin, Gyu Ri Kim

***Abstract**: The aim of the study was to examine the clinical efficacy and safety on anti-cellulite cosmetics containing high content caffeine complex (7.0% caffeine; 4.5% mixed hydrotropes; niacinamide and vanillin) and a transdermal penetrating peptide (100ppm arginine oligomer peptide, R6). In this study, dermal and subcutaneous lipid interface length measurement (Skin scanner DUB), skin roughness measurement (PRIMOS$^{\circledR}$Lite), researcher visual assessment (cellulite grading), thigh normal photo shoot (DSLR) were used. This study was conducted on 23 adult females with cellulite skin aged 20~50 years. As a result of total 22 subjects, excluding 1, used the test product containing a caffeine complex and a skin penetrating peptide 6 weeks in cellulite skin area and clinical results was summarized as followed: 1) the dermis and subcutaneous lipid interface length was statistically significantly decreased after 3 and 6 weeks using the product (p<0.05); 2) the skin roughness value of the thigh was statistically significantly decreased 3 and 6 weeks (p<0.05); 3) the cellulite grades of researcher's visual evaluation in the thigh area were statistically decreased (p<0.025) after 6 weeks; 4) subjective questionnaire evaluation scores increased significantly (p<0.025) after 3 and 6 weeks; 5) There was no skin adverse event reported after using the product during the study period. Therefore, the test product(JET-Slim Intensive lotion) containing a caffeine complex and a skin penetrating peptide is have beneficial effects in reducing the temporary cellulite in the thigh for 6 weeks.*

***Index Terms**: Anti-cellulite, Efficacy, Safety, Caffeine Complex, Transdermal Penetrating Peptide.*

I. INTRODUCTION

Cellulite, also known as gynoid lipodystrophy was characterized by orange peel, cottage cheese-type, or mattress appearance dimpling of the skin, seen most commonly on thighs and buttocks and abdomen of a woman [1, 2] and is easily observed in women after puberty by over 85%. It is a substance formed by a mixture of waste and moisture accumulated in the body. It is deeply felt nodules touching or not elastic, and colder than other parts. The pathophysiological causes of cellulite are not completely understood, but the structural, morphological and biochemical abnormalities of adipose tissue, hormones, microcirculatory disorders, obesity, stress, aging, genetic, post-inflammatory alteration, and lifestyle.As an etymology, cellulite can be defined as a localized metabolic disease of the subcutaneous tissue that affects a woman's body.It is also known as partial obesity. However, unlike obesity caused by fat accumulation of fat cells or adipocyte proliferation, cellulite is accompanied by structural changes in the dermis, fat layer and microcirculation. Also, the composition of triglycerides and free fatty acids is different in cellulite and obesity.

Querleux[3] and others [4] used a magnetic resonance imaging technique to image the subcutaneous fat layer into the dermal layer due to structural differences between the dermis and subcutaneous fat layer. The shape of the fibrous septum present between the subcutaneous fat is structurally different between men and women. The fibrous septum of the male has a reticular pattern while the female is vertically oriented, so the size of the "lobule" that forms the fat cell pouch is large. This is an easy way to penetrate the dermal layer of the adipose tissue of women and is seen as a cause of cellulite in women.Due to the accumulation of excess fat, the adipose cells become larger, pushing the blood vessels and increasing blood microcirculation and microcirculation, resulting in fine nodules.

The cellulite improvement methods [5-6] reported so far include cosmetic, aesthetic, and medicinal treatments for cellulite reducing substances, and physical treatments, topical application and drug therapy using injections, or a combination thereof.For example, endermology that helps circulation of the lymph and improves the circulation of microcirculation, mesotherapy that dissolves fat by using ingredients that can dissolve fat, ionizing therapy that penetrates drug ions into skin or tissue through current (iontophoresis), an ultrasonic method using a laser and a high frequency wave.

Generally, in the field of cosmetics, substances for improving cellulite are divided into four mechanisms [7,16-20,21,22], which are substances for improving blood flow, for inhibiting lipogenesis and helping to decompose fat, for constructing dermal and subcutaneous fat layers, for preventing the generation of free radicals.

Caffeine is used as a good compound in anti-cellulite

products because it prevents excessive accumulation of fat and is widely used as cellulite-degrading cosmetics. Caffeine is involved in lipolysis [8] by increasing cAMP levels in adipocytes and activating hormone-sensitive lipase (HSL), which leads to the degradation of triglycerides inthe lipolysis process and takes part in the reduction of cellulite. Caffeine also stimulates the draining lymph systems in fatty tissue by removing accumulated fat, toxin and unnecessary substances arising during the lipolysis process, which all together may impede the microcirculation in blood vessels and foster the emergence of cellulite.However, caffeine has a low solubility in water of 2.1% at room temperature but has a very low solubility which is significantly reduced to 0.1% at 2°C and is known to be insoluble in non-polar solvents[9]. It is limited in cosmetic formulations containing high concentrations of caffeine.In addition, transdermal delivery should be required that caffeine could penetrate to the epidermis, dermis, and subcutaneous fat in order toexhibit anti-cellulite effects. Recently, several studies have represented that cell-penetrating peptides (CPPs) can enhance the transdermal delivery of biomaterials [10-12]. Short arginine oligomer peptidesenabled transport across the epidermis, when applied topically to either mouse or human skin [13].

It was represented by the present author [14] that high concentration of caffeine aqueous solutions could be successfully prepared using mixed hydrotropes containing niacinamide and vanillin.Also, it was reported by the present author [15,16-20] that transdermal penetration was improved when high concentration of caffeine complex are contained with a short arginine oligomer (R6). In this study, we examined the clinical efficacy and safety onanti-cellulite cosmeticscontaininghigh content of caffeine complex and a transdermalpenetrating arginine peptide (R6).

II. MATERIAL AND METHODS

A. Test Formulations

The cosmetics used in this test are "JET-Slim Intensive lotion" manufactured by Dermafirm Co, Ltd., in Korea. The main ingredients contained 7.0% caffeine; 3.0% niacinamide and 1.5% vanilin as mixed hydrotropes; 0.01% ppm R6 as a skin penetrating peptide (arginine oligomer peptide). The other ingredients contained88.49% of emulsifier, oil, humectants, fragrance and deionized water in test products.

Caffeine and niacinamide were purchased from Daejung Chemicals and Metals Co., Ltd., in Korea and have a purity of at least 98.5% and 98.0%, respectively. Vanilin (purity, 99.0%) was purchased from Samchun Chemicals Co., Ltd., in Korea.

B. Study Protocol

In this study, KC Skin Research Center conducted the body efficacy evaluation according to the tenets of the Declaration of Helsinki and complied with the Guideline of Bioethics and Safety Act by the Ministry of Health and Welfare. The study was approved by the Institutional Review Board of KC Skin Research Center Co., Ltd., in Korea (KC-IRB-028).

1) Criteria for selection of subjects

This study selected voluntarily participating healthy women aged 20 to 50 who were BMI (Body Mass Index) 18 or higher and cellulite grade 1 or higher (based on Standard photograph) and the test site was set on both thighs. They were fully informed of all the information related to this human body test, and the subjects were willing to make a written consent and participated.

2) Criteria for excluding the selection of subjects

We excluded women who were pregnant or breastfeeding, who did not agree with the protocols of prevention of conception, who were allergic to cosmetics, medicines, and daily light exposure, who used the steroid contained ointment for more than 1 month to treat skin disease, who with no more than 6 months after participating in the same test, and who with identical or similar cosmetics or products on the study sites 3 months prior to this test.

3) Verification of the homogeneity of the subjects

The subjects who satisfied the criteria of selecting the subjects and those who did not have the criteria for selection exclusion were selected by homogeneity test for those who have relatively similar life environment, skin condition, skin care and cosmetics use.

4) Human body test design

The human body test was conducted for a total of 6 weeks

C. Evaluation Method

1) The Measure the length of the interface of the dermis and subcutaneous fat layer

The length of the interface of the dermis and subcutaneous fat layer on thighs was measured before using the test product, 3 weeks after using the product, and 6 weeks after using the product by Skin Scanner DUB(taberna pro medicum GmbH, Germany). The length of the interface of the dermis and subcutaneous fat layer on images were analyzed (Figure. 1) and used as temporary cellulite reduction effect assessment data. The unit of the length of the interface of the dermis and subcutaneous fat layer is in mm and the analytical value and the extent of cellulite reduction are proportional, so the smaller analytical value, the more temporary cellulite reduction effect.
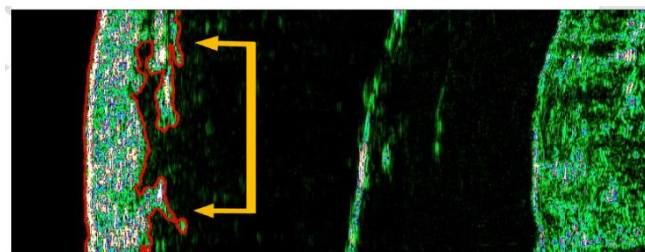

Figure 1. The length of the interface of the dermis and subcutaneous fat layer

2) The skin roughness measurement

The skin roughness was measured using PRIMO lite (LMI Technologies GmbH, Canada) on thighs before using the product, 3 weeks after using the product, and 6 weeks after using the product. The photographed images were analyzed by using PRIMOS-specific software to determine the volume of cavities on site. The unit of the skin roughness analysis value is mm^3, and the analytical value is inversely proportional to the skin roughness improvement degree, which means that the skin roughness is improved as the analysis value is decreased.

3) Photographs of thighs and visual evaluation of the researchers

The thigh photographs were taken on the left sides of the subject's thighs at the same conditions (before use, after 3 weeks of use, and after 6 weeks of use) at each visit using a high-resolution digital camera (DSLR: Canon Inc, Taiwan). The visual evaluation of the researchers was carried out by two researchers according to the criteria of cellulite grade (Figure 2). Before the test product was used, a visual evaluation by the researchers was performed to determine whether the selection criterion was appropriate (cellulite grade 1 or higher) and checked the change in the grade after 3 weeks using the product and 6 weeks using the product. If the researchers had different assessments, the low-grade was chosen.
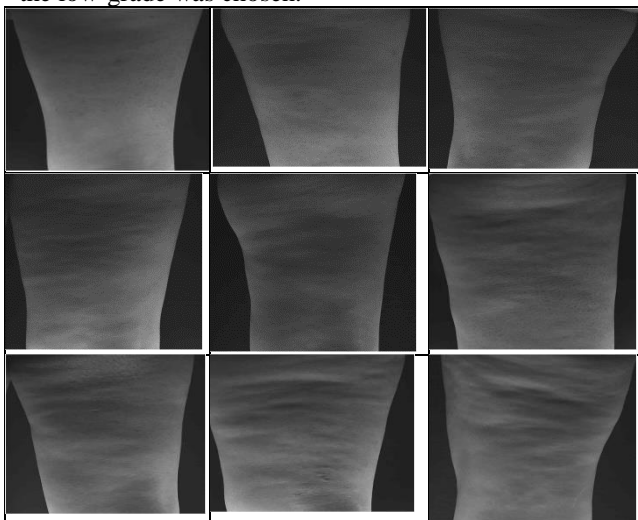

Figure 2. The grade of cellulite
(1~9: mild intensity ~ severe intensity)

4) Measurement of the circumference of the thighs
The circumference of the left and right thighs was measured by InBody Co., Ltd., Korea) before using the product, 3 weeks after using the product, and 6 weeks after using the product. The unit of the circumference of the measured thighs is cm. The measured value and the circumference of the thighs are proportional, so the change in the thigh circumference according to the degree of cellulite reduction can be seen.

5) Evaluation of skin adverse reaction
If the test site shows any adverse reactions or other symptoms such as erythema, edema, stinging, burning, tightness, prickling, or irritability, it is stated on individual case report form (CRF) with degree of the symptom: mild, moderate, or severe.

III. RESULTS AND DISCUSSION

A. Results of the dermis and subcutaneous fat layer interface measurement

The changes in the dermis and subcutaneous fat layer were measured 3 times, including before application (0 weeks), after 3 weeks, and after 6 weeks. Measurement of its layerafter use of the test product showed a decrease to 22.035 ± 3.676 mm after 3 weeks and 21.865 ± 3.875 mm after 6 weeks, from 21.786 ± 3.736 mmbefore product use(Table 1).

To analyze the rates of improvements, when the degrees of change on the 3nd and 6th week were 100% based on the degree of the interface length before the use of the product, the degree of its length decreased by 3.239% after 3 weeks, 4.079% after 6 weeks (Table 1). Therefore, it was concluded that as results of measuring the interface length of the dermis and the subcutaneous fat layer of the thighs, it was statistically significantly decreased after 3 weeks and 6 weeks using the product ($p<0.05$).

Table 1. Results of the length of the interface of dermis and subcutaneous fat layer measurements

Time	Average ± STD (mm)	Improvement rate[a](%)	Probability [b] (p value)
luct use	22.786±3.736	-	-
After 3 weeks	22.035±3.676	-3.239	0.007 **
After 6 weeks	21.865±3.875	-4.079	0.005 **

• Improvement rate[a] (%) = [(After product use –Before product use) / Before product use] x 100
•Probability[b] (p value) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

B. Results of the skin roughness measurement

The changes in the skin roughness were measured 3 times, before using the product (week 0), after 3 weeks, and after 6 weeks. The results showed that the skin roughness decreased from 170.308 ± 25.050 to 164.100 ± 22.839 after 3 weeks and to 162.507 ± 21.702 after 6 weeks (Table 2, $p<0.05$).

The degrees of the improvement in the 3rd and 6th weeks were calculated as percentage to analyze improvement rate, by setting the skin roughness after using test product as 100%. Skin roughness decreased by 3.461% after 3 weeks, while skin roughness decreased by 4.297% after 6 weeks (Table 2). From these results, it was found that as results of measuring the skin roughness value of the thigh, it was statistically significantly decreased after 3 and 6 weeks ($p<0.05$).

Table 2. Results of the skin roughness measurement

Time	Average ± STD (mm^3)		Probability^b (p value)
luct use	170.308±25.050	-	-
After 3 weeks	164.100±22.839	-3.461	0.000 **
After 6 weeks	162.507±21.702	-4.297	0.000 **

• Improvement rate^a (%) = [(After product use – Before product use) / Before product use] x 100

•Probability^b (p value) **: p<0.05 by Repeated measured ANOVA, post hoc Bonferroni correction

C. Results of cellulite grade

Changes in cellulite grade were assessed 3 times, including before using the test product, after 3 weeks, and after 6 weeks of using the test product. Using the test product resulted in a decrease of its index from 3.045 ± 1.397 before use, to 2.773 ± 1.307 after 3 weeks and to 2.409 ± 1.182 after 6 weeks.

Analysis of the rate of improvement in cellulite grade revealed that the percentage of change between the 3nd and 6th weeks was 100% and the extent of cellulite grade decreased by 7.348% after 3 weeks and20.152% after 6 weeks. Figure3 shows clinical pictures of cellular grade measurement of major subjects using DSLR.Therefore, it was concluded that asresults of cellulite grade on the thighs after using test product, the visual evaluation by researchers was statistically reduced 6 weeks (Table 3, p<0.05), after using the product compared to before use.

Table 3. Results of cellulite grade

Time	Average ± STD (mm)	Improvement rate^a(%)	Probability^b (p value)
luct use	3.045 ± 1.397	-	Effects in individual subjects 0.000
After 3 weeks	2.773 ± 1.307	-7.348	0.034
After 6 weeks	2.409 ± 1.182	-20.152	0.000†

• Improvement rate^a (%) = [(After product use –Before product use) / Before product use] x 100

•Probability^b (p value) †: p<0.025(5%/2) by Friedman test, post hoc Wilcoxon signed rank test

Table 3. Results of cellulite grade
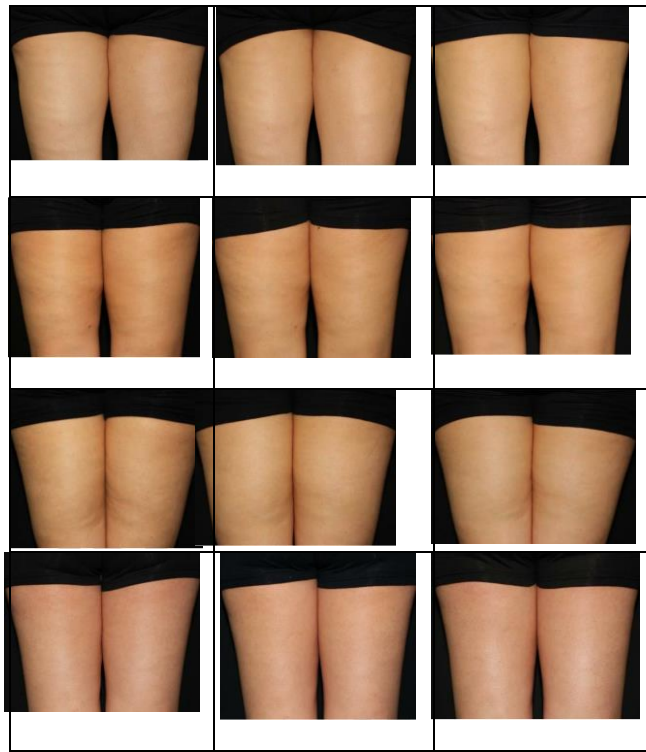
(a) Before product use   (b) After 3 weeks   (c) After 6 weeks

Figure 3.Results of cellulite grade (DSLR)

D. The results of the measurement of the circumference of the thighs

As results of the measurement of the circumference of the thighs after using the product(Table 4)., this measurement results after 3 weeks and 6 weeks of product use showed no statistically significant difference (p<0.05).

Table 4. The change of the circumference of the thighs

Time	Average ± STD (cm)	Improvement rate^a(%)	Probability^b (p value)
luct use	53.582±2.598	-	-
After 3 weeks	53.714±2.548	0.261	1.000
After 6 weeks	53.286±2.451	-0.530	0.260

• Improvement rate^a (%) = [(After product use –Before product use) / Before product use] x 100

•Probability^b (p value) **: p<0.05 by Repeated measured ANOVA, post hoc Bonferroni correction

E. Evaluation of skin adverse reactions

In the test subjects, the presence of adverse skin reactions such as erythema, edema, scaling, itching, stinging, burning, tightness, ting (rickets, swelling, scurvy, itching, aching, burning, stiffness, tingling) among others was investigated every time subject presented themselves for analysis. No specific skin adverse events were observed in all subjects participating that participated in the present study (Table 5).

Table 5. Assessing skin adverse events

Time	Erythema	Edema	Scaling	ng
After 3 weeks	-	-	-	-
After 6 weeks	-	-	-	-
Time	Stinging	Burning	Tightness	Prickling
After 3 weeks	-	-	-	-
After 6 weeks	-	-	-	-

Step=1: Weak, 2: Medium, 3: Severe

IV. CONCLUSIONS

In this research, the clinical efficacy and safety was studied on anti-cellulite cosmetics containing 7.0% high content caffeine, 4.5% mixed hydrotropes, (niacinamide and vanillin) and100ppm transdermal penetrating peptide (arginine oligomer peptide, R6). A total of 23 subjects who met the criteria for selection and who did not meet the exclusion criteria and who were consenting to participate in the human body test were used for 6 weeks at the designated site. After 6 weeks of use, the photographs and skin measurements were taken before use and 3 weeks after use and 6 weeks after use. Subjects were asked to wash with their skin the same cleanser and after 30 minutes of stabilization in an indoor environment maintained at constant temperature and humidity. In this study, dermal and subcutaneous lipid interface length measurement (Skin scanner DUB), skin roughness measurement (PRIMOS®Lite), researcher visual assessment (cellulite grading), thigh normal photo shoot (DSLR) were used. This study was conducted on 23 adult females with cellulite skin aged 20~50 years. As a result of total 22 subjects, excluding 1, used the test product containing a caffeine complex and a skin penetrating peptide 6 weeks in cellulite skin area and clinical results was summarized as follows: 1) the dermis and subcutaneous lipid interface length was statistically significantly decreased after 3 weeks and 6 weeks using the product ($p<0.05$); 2) the skin roughness value of the thigh was statistically significantly decreased 3 weeks and 6 weeks using the product ($p<0.05$); 3) the cellulite grades of researcher's visual evaluation in the thigh area were statistically decreased ($p<0.025$) after 6 weeks of using the product; 4) subjective questionnaire evaluation scores increased significantly ($p<0.025$) after 3 weeks and 6 weeks using the product use; 5) There was no skin adverse event reported after using the product during the study period. Therefore, the test product (JET-Slim Intensive lotion) containing a caffeine complex and atransdermal penetrating peptide is have beneficial effects in reducing the temporary cellulite in the thigh for 6 weeks.

ACKNOWLEDGMENT

REFERENCES

1. Z. Draelos, K.D. Marenus KD, "Cellulite. Etiology and purported treatment", *Dermatol. Surg.*, Vol. 23, 1997, pp. 1177-1181.
2. G.E. Pierard, J.L. Nizet, C. Pierard-Franchimont C, "Cellulite: from standing fat herniation to hypodermal stretch marks", *Am. J. Dermatopathol.*, Vol. 22, 2000, pp. 34 –37.
3. B. Querleux, C. Cornillon, O. Jolivet, J. Bittoun, "Anatomy and physiology of subcutaneous adipose tissue by in vivo magnetic resonance imaging and spectroscopy: relationships with sex and presence of cellulite", *Skin Res. Technol.*, Vol. 8, 2002, pp. 118–124.
4. F. Nurnberger, G. Muller, "So-called cellulite: an invented disease", *J. Dermatol. Surg. Oncol.*, Vol, 4, 1978, pp. 221–229.
5. M.P. Goldman, "Cellulite: a review of current treatments", *Cosmet. Dermatol.*, Vol. 15, 2002, pp. 17–20.
6. M.M. Avram, "Cellulite: a review of its physiology and treatment", *J. Cosmet. Laser Therapy*, Vol. 6, 2004, pp. 181–185.
7. E.L. Sainio, T. Rantanen, L. Kanerva, L. "Ingredients and safety of cellulite creams", *Eur. J. Dermatol.*, Vol. 10, 2000, pp. 596-603.
8. A. Herman, A.P. Herman, "Caffeine's mechanisms of action and its cosmetic use", *Skin Pharmacol. Physiol.*, Vol. 26, 2013, pp. 8-14.
9. L. R. Morrison Jr., M. N. Elder, J. H. Phillips, "Recovery of noncaffeine solubles in an extract decaffeination process", *EU Patent,* 19820200206,1982.
10. H. Kamada, T. Okamoto T, M. Kawamura, H. Shibata, Y. Abe, A. Ohkawa, T. Nomura, M. Sato, Y. Mukai, T. Sugita, S. Imai, K. Nagano, Y. Tsutsumi, S. Nakagawa, T. Mayumi, S. Tsunoda, "Creation of novel cell-penetrating peptides for intracellular drug delivery using systematic phage display technology originated from Tat transduction domain", *Biol. Pharm. Bull.*, 30, 2007, pp. 218-223.
11. Y.C. Kim, P.J. Ludovice, M.R. Prausnitz, "Transdermal delivery enhanced by magainin pore-forming peptide", *J. Control. Release*,Vol. 122, 2007, pp. 375–383.
12. J. Lee, E. Jung, J. Park, D. Park, "Transdermal delivery of interferon-gamma (IFN-gamma) mediated by penetratin, a cellpermeable peptide",*Biotechnol. Appl. Biochem.*, Vol. 42, 2005, pp. 169-173.
13. J.B. Rothbard, S. Garlington, Q. Lin, T. Kirschberg, E. Kreider, P.L. McGrane, P.A. Wender, P.A. Khavari., "Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation", *Nat. Med.*, Vol. 6, 2000, pp. 1253-1257.
14. Castilla, A. S., Hernández, R. M., & Rodríguez, J. M. A. The Importance of Clinical Genotype in Patients with Klinefelter Syndrome. A Genetic Disorder Asociated to Taurodontism. Journal of Diseases, 2(2), 12-22, 2015.
15. M.S. Shin, "Anti-cellulite cosmetic composition of aqueous caffeine complex with mixed hydrotropes and transdermal penetrating peptide (R6)", *Korean Patent Application*, 10-2019-0015302, 2019.
16. Ali, A., & Haseeb, M. (2019). Radio frequency identification (RFID) technology as a strategic tool towards higher performance of supply chain operations in textile and apparel industry of Malaysia. Uncertain Supply Chain Management, 7(2), 215-226.
17. Awang, Z., Ahmed, U., Hoque, A. S. M. M., Siddiqui, B. A., Dahri, A. S., and Muda, H. (2017). The Mediating Role of Meaningful Work in the Relationship Between Career Growth Opportunities and Work Engagement, International Academic Confernce on Business and Economics (IACBE 2017), Faculty of Economics and Management Sciences (FESP), Universiti Sultan Zainal Abidin (UniSZA), October 07-08
18. Haseeb, M., Abidin, I. S. Z., Hye, Q. M. A., & Hartani, N. H. (2018). The Impact of Renewable Energy on Economic Well-Being of Malaysia: Fresh Evidence from Auto Regressive Distributed Lag Bound Testing Approach. International Journal of Energy Economics and Policy, 9(1), 269-275.
19. Haseeb., H. Z., G. Hartani., N.H., Pahi., M.H. Nadeem., H. . (2019). Environmental Analysis of the Effect of Population Growth Rate on Supply Chain Performance and Economic Growth of Indonesia. Ekoloji, 28(107).
20. Suryanto, T., Haseeb, M., & Hartani, N. H. (2018). The Correlates of Developing Green Supply Chain Management Practices: Firms Level Analysis in Malaysia. International Journal of Supply Chain Management, 7(5), 316.
21. Prayogo, L., Pranoto, B., & Purba, H. (2017). Employee satisfaction analysis with human resource index. Management Science Letters, 7(5), 233-240.
22. Bahadoran, H., & Nazari, M. (2018). Investigating the effect of empowerment aspects on the competence level and success of primary school principals. Management Science Letters, 8(5), 445-454.