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Red Meniran : The Secret to Minimizing Exposome-Induced Skin

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1. Introduction

The Exposomes concept is an accumulation of exposures from variety of external and internal sources including chemical agents, biological agents, or radiation, from conception onward, over a complete lifetime [1]. Exposomes which continuously expose the skin in the daily life can impact the skin conditions, in result the skin shows imperfect appearance by inducing or inducing the exposomes. Several skin imperfections could happen because of UV exposure, pollution, and sleep deprivation. Sun-exposed skin shows more aging-skin features, wrinkles, dullness, pigmentary changes, and roughness. Sun exposures can also increase the production of oxidative stress, skin pigmentation would be unavoidable and known as UVB-induced skin pigmentation [2]. The role of UV radiation in skin aging is well established and the term photoaging has been coined to emphasize this cause-and-effect relationship. UV radiation is considered as the environmental factors contributing to the exposome of skin aging [3].

Another skin photoaging exposome contributor is Air pollution, the pollution is a contamination of either from indoor or outdoor environment by any chemical, physical or biological agent [3]. Poor sleep quality has also been related to skin barrier impairment. It is caused by an occasional drop in oxygen saturation due to repeated interruptions or a reduction in airflow and upper airway obstruction [4]. These facfors leading to the disruption of skin barrier, erythema, and acne.

Nowadays people are looking for chemical or cosmetics ingredients that do no harm to the skin or any other parts of the body. Indonesia knowns for the “mega-biodiversity” title since it has a lot of biodiversity and located in the unique geographic that makes it inhabited the varieties ecosystem of endemic or native plants [5]. The clean beauty trend and Indonesia’s mega-biodiversity emphasizes this study to explore for a new natural ingredient from Indonesia native plant.

Phyllanthus urinaria is commonly used for treatment of fever, improving eyesight, urinary problems, and liver diseases and do detoxify posion from the body [6]. *P. urinaria* is also known to have bioactive compounds such as coumarin, ellagitannin, and sterol that can act as antioxidant, antiviral, and anti-inflammatory [6]. Dayak Tribe in Kalimantan, Indonesia also use Red Meniran as traditional medicine to treat herpes zoster. It was called “Uri Handalai Bahandang”. The ethanolic extract of *P. urinaria* is reported to have strong antioxidant activity and envisaged that the metabolites compounds in *P. urinaria* extract might prevent the oxidation stress and thus inhibit the damage caused by UV exposures [7]. This study was conducted to explore the potency of Red Meniran Extract (RME) as cosmetic ingredients to solve skin problems affected by exposome.

2. Materials and Methods

Materials

Red meniran leaf (*P. urinaria)* sample was collected from local supplier in Blora, Central Java, Indonesia. Ethanol 96%. 2,2-dipheynyl-1-picrihydrazyl (DPPH) reagents. Mushroom derived tyrosinase, potassium phosphate buffer, L-DOPA, *Cutibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 6538, and *Staphylococcus epidermidis* ATCC 12228. Cell line B16F10, arbutin, alpha-melanocyte stimulating hormone (α-MSH), Keratinocyte (HaCaT p.18), Dimethylsulfoxide (DMSO), dexamethasone, Polyinosinic:polycytidylic acid (Poly I:C), Interleukin-4 (IL-4). Sodium lauryl sulfate (SLS) patch, tyrosinase enzyme. Purified water, 1,3-butylene glycol, 1,2-hexanediol.

Preparation of Red Meniran Extract *for antioxidant* assay

The RME extract, serving as the primary sample for this study, was prepared according to the procedures outlined in the Indonesia Herbal Pharmacopoeia. Extraction was performed by maceration over a period of three days, with daily replacement of the 96% ethanol solvent. The liquid extract obtained was subsequently concentrated by evaporating the solvent using a rotary evaporator.

Antioxidant activity assay using DPPH method

The DPPH assay was employed to evaluate the antioxidant activity of the RME. A DPPH solution was prepared by dissolving DPPH at a concentration of 50 µg/mL in methanol, while a positive control solution was prepared by dissolving ascorbic acid at a concentration of 200 µg/mL in methanol. The initial Absorbance (A_0) of the DPPH solution was measured at 517 nm using a UV-visible spectrophotometer. A stock solution of the extract was prepared at a concentration of 500 µg/mL, followed by serial dilutions in methanol to obtain various concentrations. Each sample dilution was mixed with 0.5 mL of the DPPH solution, sealed and incubated in the dark for 30 minutes. The absorbance of each mixture was then measured at 517 nm with a UV-visible spectrophotometer. A blank solution consisting of methanol was used to establish a baseline. The antioxidant activity of the samples was expressed in terms of the IC_{50} value.

Anti-inflammatory and anti-itching assay

The expression levels of IL-1β mRNA were measured using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Total RNA was isolated from HaCaT cells with the RNeasy Plus Kit (Qiagen, CA, USA) in accordance with the manufacturer’s guidelines. To induce an inflammatory response, HaCaT cells at passage 13 (p13) were treated with Poly I:C (10 µg/mL) and IL-4 (10 µg/mL). Following induction, cells were exposed to cinnamon bark

extract at concentrations of 1 µg/mL and 10 µg/mL, or to dexamethasone (1 µM) as a positive control, for four hours. Statistical comparisons were conducted using a one-way analysis of variance (ANOVA) and two-tailed unpaired Student's t-test.

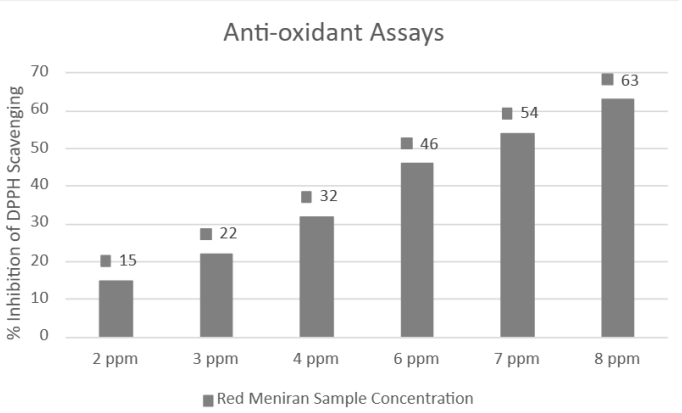
In vivo test of skin sebum amount and skin soothing

The clinical study was conducted on 22 volunteers (average age of 31.32±9.55 years) with the Red Meniran serum with the concentration of 3%. The serum was applied twice a day, in the basic care step after washing the fase, the product applied for appropriate amount of the RME to the right facial area.

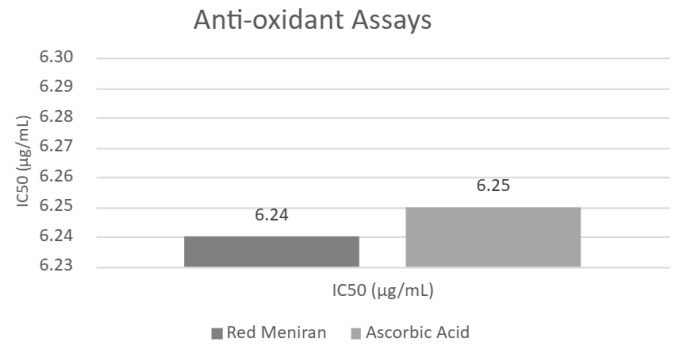
The skin sebum amount was analyzed by average percentage (%) value of the sebum spot area on the left or right cheek areas from the UV captured image using the device's own analysis software. Skin redness was evaluated as the parameter skin soothing effect. The parameter was measured three times each of the facial area to analyze the mean value at before and after 4 weeks of treatment.

3. Results

Antioxidant activity of RME in IC_{50} value



(a)



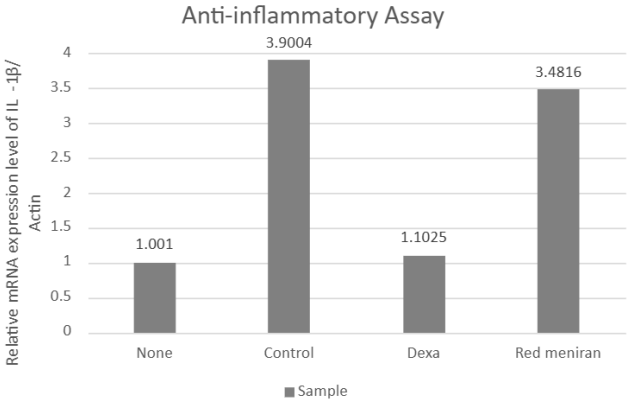
(b)

Figure 1: Antioxidant activity of Red Meniran Extract: (a). % Inhibition of DPPH scavenging; (b) IC_{50} comparison between Red Meniran Extract and Positive Control (Ascorbic Acid)

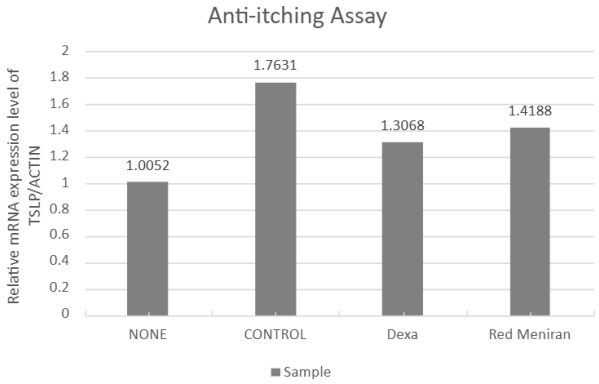
Based on the results, RME exhibited an increased percentage of DPPH scavenging activity in a concentration-dependent manner, as illustrated in **Figure 1a.** The data were then plotted to obtain the corresponding linear equation and calculate the IC_{50} value of RME. RME showed antioxidant value of IC_{50} **6.24 µg /mL.** Ascorbic acid as the positive control had IC_{50} **6.25 µg /mL** after measured by DPPH method.

Expression of IL-1β and TSLP in HaCaT cells

The anti-inflammatory and anti-itching properties of RME were evaluated using HaCaT cells. In this study, IL-1β expression levels were used as markers of anti-inflammatory activity, while TSLP expression served as indicator of anti-itching activity. The positive control that were used in this study is dexamethasone which is a drug that has an anti-inflammatory and anti-itching effect. The cell line was induced by Poly I:C 10 and IL – 4. Figure **2a and Figure 2b** shows the treatment of RME can reduce the relative mRNA expression level of IL-1β and TSLP. It was indicated that RME has anti-inflammation and anti-itching effect.



(a)



(b)

Figure 2: Relative expression level o Poly I:C and IL-4-induced HaCaT cells: (a). IL- *β expression*; (b). TSLP Expression

Skin Sebum Amount Evaluation

Skin sebum level was used to indicate sebum regulation activity of RME. Based on in vivo test result, RME 3% serum showing a significant decreased of skin sebum amount after 4 weeks of treatment ($p<0.05$) comparing before and after treatment. The comparison between groups, the group that were applied RME 3% Serum was 14.15% change rate and significantly decreased more than the control or placebo group, which was 4.87%. (**Table 1 and 2; Figure 3 and 4)**

Skin-soothing efficacy of RME

Skin Redness were evaluated to see the skin soothing effect of RME. The result showed that RME 3% serum significantly reduced skin redness (a* value) after 4 weeks ($p<0.05$) comparing between before and after treatment. Compared to control groups, the group that were applied RME 3% serum was 12.66% change rate and significantly decreased more than control group, which was 8.48%.

Table 1. Stastical analysis of before/after comparison results for skin sebum

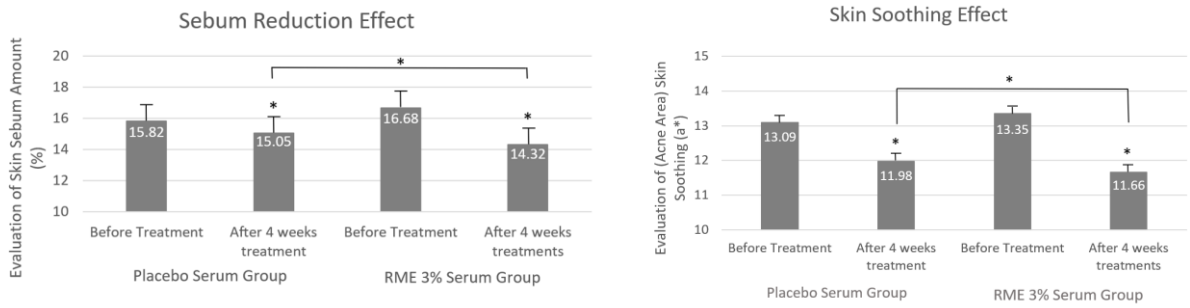
Group	Time Point	*p-value*	Change rate (%)
RME 3%	Before Treatment		
	After 4 weeks treatment	0.000*	**14.15**
Anti Acne Serum (Control)	Before Treatment		
	After 4 weeks treatment	0.144	4.87

p<0.05, there is significant difference

Table 2. *S*tastical analysis of before/after comparison results for (acne area) skin soothing

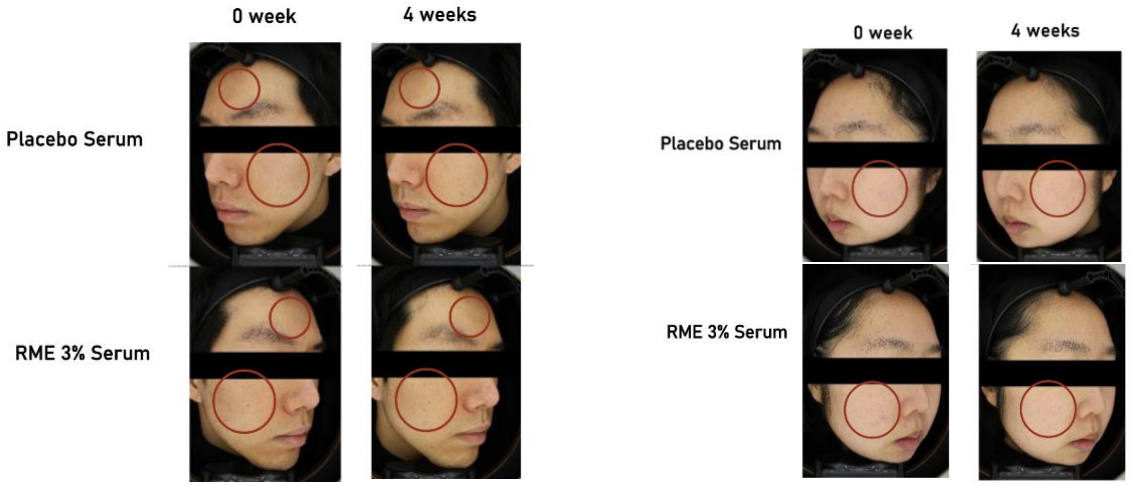
Group	Time Point	*p-value*	Change rate (%)
RME 3%	Before Treatment		
	After 4 weeks treatment	0.000*	**12.66**
Anti Acne Serum (Control)	Before Treatment		
	After 4 weeks treatment	0.000*	8.48

p<0.05, there is significant difference



(a) (b)

Figure 3: Skin-soothing efficacy for Red Meniran Extract: (a) Sebum Reduction Effect; (b) Skin Soothing Effect



(a) (b)

Figure 4: In Vivo Skin-soothing efficacy: (a) Comparison for Skin Sebum Reduction; (b) Comparison for Skin Soothing Effect

4. Discussion

The in vitro and in vivo exploration on RME were conducted to see the potency as cosmetic ingredients. Based on DPPH assay, RME has antioxidant activity. IC_{50} was calculated to classify the antioxidant capacity of RME. IC50 is the inhibition concentration of sample to reduce 50% of free radicals. Antioxidant activity of RME was evaluated through its IC_{50} value, which indicates the concentration required to neutralize 50% of free radicals. According to IC_{50} measurements, compounds are classified as very strong antioxidants if the value is below 50 µg/mL, strong if between 50–100 µg/mL, moderate if between 101–150 µg/mL, and weak if the value exceeds 150 µg/mL [8]. RME showed a very strong antioxidant activity with IC_{50} value 0f 6.24 µg/ml, compared to the positive control Ascorbic acid with IC_{50} value 0f 6.25. This shows that the antioxidant activity of RME has an anti-oxidative effect by increasing DPPH radical scavenging ability in a dose dependent manner [9] (Natraj *et al*., 2018). RME exhibits considerable

free-radical-scavenging activity in the 1,1-diphenyl-2-picrydydrazyl (DPPH)-assay [10]. The pigments that play an important role in antioxidant ability in the *P. urinaria* plant are chlorophyll and carotenoids which allow them to capture free radicals and reduce oxidative stress [11]. The biochemical compounds that isolated from *P. urinaria* extract could minimize the oxidative stress and are considered as an alternative source of medicine for skin aging amelioration [12].This suggests that RME was efficient as antioxidant agent for ROS scavengers in the skin.

From antiiflammation and anti-itching assay, RME decreased the amount of mRNA expression of IL-1β and TSLP. Interleukin-1β is a pro-inflammatory mediator that triggers the onset of inflammation, and its dysregulated signaling contributes to the development of various acute and chronic diseases. [13].Thymic stromal lymphopoietin (TSLP) is a cytokine associated with itch, especially in disorders such as atopic dermatitis (AD). It is secreted by epithelial cells and contributes to the development of allergic diseases by triggering itch and stimulating sensory neurons [14]. Inflammatory responses in the skin trigger the release of various mediators such as histamine, cytokines (IL-1, TSLP) that can directly activate sensory neurons responsible for the itch sensation [15]. Previous study showed that isolated compound from *P. urinaria* Herb such as phyllanthin, phyltetralin, trimethyl-3,4-dehydrochebulate, methylgallate, rhamnocitrin, methyl brecifolincarboxylate, quercitrin, and rutin can inhibit the production of pro-inflammatory factor such as TNF-α, IL-6, IFN-γ dose dependently [16]. *P. urinaria* leaves also contain flavonoid and tannins that have antiinflammation properties [12]. Until this day the evaluation of *P. urinaria* leaf extract against the thymic stromal lymphopoietin activity (TSLP) are still undefined, RME shows a reduction of relative mRNA expression level of TSLP compared to the control. *P. urinaria* have the immunomodulator activity through signaling pathways such as NF-kB, JAK/STAT, and MAPK [17]. These pathways are known to play a role in regulation TSLP expression, which is involved in various inflammatory and allergic conditions such as itching [18. Based on the in vitro test results showed that *P. urinaria* or Red Meniran Extract (RME) has antiinflammation and antiitching properties.

From the clinical evaluation, the 3% RME serum shows a reduction of sebum production after 4 weeks of treatment. In comparison *Phyllanthus emblica* which coming from the same genus as *Phyllanthus urinaria* demonstrated significant sebum reduction. This could demonstrated that the two specieses share some phytochemical constituents, such as tannins, which is known for its astringent and antioxidant properties [19]). The sared compounds might suggest a potential similarities in their effects on sebum production. RME also indicated soothing effect. RME 3% serum can significantly decrease skin redness compared to control group. Several flavonoid and tannins compounds such as rhamnocitrin, rutin, quercetin, naringin epigallocathechin, epicatechin-3-gallate play a role in decreasing pro-inflammatory mediators production. Inhibition of inflammatory factor production leading to reduction of skin redness [12].

5. Conclusion

In conclusion, this study, wich included several in vitro and clinical evaluation, confirmed that *P. urinaria* Extract from Indonesia offers good efficacy for the skin as cosmetic ingredients. The results highlight its multifunctional properties including sebum reduction and soothing effect.

References

1. Vineis, P., Robinson, O., Chadeau-Hyam, M., Dehghan, A., Mudway, I., & Dagnino, S. (2020). What is new in the exposome? Environment International, 143(February), 105887. https://doi.org/10.1016/j.envint.2020.105887
2. Passeron, T., Krutmann, J., Andersen, M. L., Katta, R., & Zouboulis, C. C. (2020). Clinical and biological impact of the exposome on the skin. Journal of the European Academy of Dermatology and Venereology, 34(S4), 4–25. https://doi.org/10.1111/jdv.16614
3. Krutmann, J., Boulouc, A., Sore, G., Bernard, B. A., Passeron, T. (2016). The Skin Aging Exposome. *Journal of Dermatological Science*. https://doi.org/10.1016/j.jdermsci.2016.09.015
4. Vilchez, R, M., Vilchez, T, M., Fernandez, M., Pozo, J., Galvez, G, J., Garcia, C, M., Eisman, A, B., Santiago, S, A. (2022). Impact of Exposome Factors on Epidermal Barrier Function in Patients with Obstructive Sleep Apnea Syndrome. *International Journal of Environmental Research and Public Health*. 19(2), 659. https://doi.org/10.3390/ijerph19020659
5. Setiadi, A., Adieb Pritanto, A., Sri, B., Alhumaira, F., & Khasanah, S. N. (2023). Konservasi Keanekaragaman Hayati Endemik Melalui Ecology, Socio-Economic, Dan Socio-Cultural Approach (Studi Pada Taman Kehati Kokolomboi, Sulawesi Tengah). Jurnal CSR, Pendidikan, Dan Pemberdayaan Masyarakat, 4(1), 244–254.
6. Zain, S., Omar, W. (2018). Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content of Water and Methanol Extracts of *Phyllanthus* species from Malaysia. *Parhmacogn*. 10(2). DOI : 10.5530/pj.2018.4.111
7. Arsiningtyas, I, S., Simamora, I, S., Palimbongan, A, M., Widianingtyas, F, D., Junedi, S. (2024). In Vitro Anti-Photoaging Properties of *Phylanthus urinaria* L. Herb Extract. *Indones. J. Cancer Chemoprevent*. 15(1). e-ISSN: 2355-8989
8. Sukweenadhim J. (2020). Antioxidant activity screening of seven Indonesian herbal extract. *Biodiversitas*. 21(5). Doi: 10.13057/biodiv/d21
9. Natraj, P., Yang, G., Moon, H., Han, C. (2018). Anti-oxidant, anti-bacterial, and anti-inflammatory effects of *Phyllanthus urinaria* L.
10. Xu, M., Zhu, Z., Qin, X., Zhang, X., Yang., Zhang, Y. (2007). Phenolic Antioxidants from the Whole Plant of *Phyllanthus urinaria*. *Chemistry & Biodiversity*. 4.
11. Araujo, O, F., Kristiani, E., Kasmiyati, S., Nugroho, L. (2024). The Phytochemical and Biological Activities of Two *Phyllanthus* Species : Insights into Metabolit, Antioxidant and Antibacterial Activity. *Biosaintifika*. 16(3). p -ISSN 2085-191X | e-ISSN 2338-7610

12. Du, G., Xiao, M., Yu, S., Wang, M., Xie, Y., Sang, S. (2018). *Phyllanthus urinaria* : a potential phytopharmacological source of natural medicine. *Int J Clin Exp Med.* 11(7).
13. Paolo, N, C., Shayakhmetov, D, M. (2016. Interleukin 1α and the inflammatory process. *Nat Immunol*. 17(8). Doi: 10.1038/ni.3503.
14. Wilson, S, R. The., Batia, L, M., Beattie, K., Katibah, G, E., McClain, S, P., Pellegrino, M., Estandian, D, M., Bautista, D, M. (2013). The Epitheal Cell-Derived Atopic Dermatitis Cytokine TSLP Activates Neurons to Induce Itch. *Cell*. 155.
15. Wong, L, S., Wu, T., Lee, C, H. (2017). Inflammatory and Noninflammatory Itch : Implications in Pathophysiology-Direceted Treatments. *Int J Mol Sci.* 18(7). Doi : 10.3390/ijms18071485
16. Fang, S, H., Rao, Y, K., Tzeng, Y. (2008). Anti-oxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from *Phyllanthus urinaria*. *Ethnopharmacol.* 116(2). Doi : 10.1016
17. Liu, L., Wang, B., Ma, Y., Sun, K., Wang, P., Li, M., Dong , J., Qin, M., Li, M., Wei, C., Tang, Y., He, J., Guuo, K., Yu, X. (2024). A review of *Phyllanthus urinaria* L. in the treatment of liver disease : viral hepatitis, liver fibrosis/ cirrhosis and hepatocellular carcinoma. *Frontiers in Pharmacology*. DOI 10.3389/fphar.2024.1443667
18. Lee, H., *and* Ziegler, S. (2007). Inducible expression fo the proallergic cytokine thymic stromal lymphopoietin in airway epithelial celss is controlled by NFkB. *PNAS.* 104(3).
19. Timudom, T., Chaiyasut, C., Sivamaruthi, B, S., Tiampasook, P., Nacapunchai, D. (2020). Anti-Sebum Efficacy of *Phyllanthus emblica* L. (Emblica) Toner on Facial Skin. *Applied Science.*