MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
e-ISSN 2774-2318
p-ISSN 2407-0505

Research Article
MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
Journal Page: https://jurnal.unimus.ac.id/index.php/APKKM

Spray Preparation Nanoemulsion Containing Butterfly Pea Flower (Clitoria ternatea L) Ethanol Extract as an Antiaging Nanocosmetic

Lelly Yuniarti[1], Tryando Bhatara[2], Maya Tejasari[3], Miranti Kania Dewi[4], Sadiah Ahmad[1], Yuktian Kharisma[5]

[1)]Department of Biochemistry, Nutrition, and Biomolecular, Faculty of Medicine, Universitas Islam Bandung, West Java, Indonesia
[2)]Department of Biomedicine, Faculty of Medicine, Universitas Islam Bandung, West Java, Indonesia
[3)]Department of Histology, Faculty of Medicine, Universitas Islam Bandung, West Java, Indonesia
[4)]Department of Pharmacology, Faculty of Medicine, Universitas Islam Bandung, West Java, Indonesia
[5)]Department of Anatomical Pathology, Faculty of Medicine, Universitas Islam Bandung, West Java, Indonesia

Article Info

Article history:
Received 29 November 2023
Revised 19 July 2024
Accepted 12 August 2024
Available online 18 August 2024

Keywords:
Antiaging; Clitoria Ternate L; elastin; nanoemulsion spray; collagen

Correspondent:
lelly.yuniarti@gmail.com

How to cite this article:

Lelly Yuniarti, Tryando Bhatara, Maya Tejasari, Miranti Kania Dewi, Sadiah Ahmad, Yuktian Kharisma., Spray preparation nanoemulsion containing butterfly pea flower (Clitoria ternatea L) ethanol extract as an antiaging nanocosmetic. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2024; 11(2): 189-197

Abstract

Background: Nanotechnology has the potential to produce innovations in drug formulation and drug delivery systems. Nanocosmetics and nanocosmeceuticals containing natural ingredients have been proven to overcome traditional cosmetics' weaknesses and add value to their formulations.

Objective: This study aims to analyze the application of nanocosmeceutical technology based on ethanol extract of butterfly pea flower (Clitoria ternatea L) in the preparation of nanoemulsion spray as a skin antiaging agent for the growth of collagen, elastin, and extracellular matrix.

Methods: A true experimental design was used with a posttest control group with random allocation to model mice given UVB radiation. The levels of collagen, elastin, and cellular matrix extract were assessed using Verhoef staining and observed by two experts,

Results: The results of this research show that a nanoemulsion spray preparation of ethanol extract of butterfly pea flower (Clitoria ternatea L) (NESCT) improves skin damage in aging model mice by increasing the production of extracellular matrix, collagen, and elastin.

Conclusion: The preparation of butterfly pea flower extract nanoemulsion spray (Clitoria ternate L) has antiaging, collagen and elastin formation activities

e-ISSN 2774-2318
p-ISSN 2407-0505

INTRODUCTION

Nowadays, skin aging has become a matter of concern to people, particularly women.[1] The aging process is marked by an accumulation of macromolecular disruption within the cell, declining regenerative capabilities of tissues, and inability to restore physiological integrity.[2] The most observable manifestation of skin aging is a cumulative change regarding skin structure, drying skin, dysfunctional and decline in skin appearance such as increased wrinkles, weakness, elastosis, telangiectasia, and inappropriate skin pigmentation3 and increased risk of skin diseases.[4] Skin aging is not only influenced by age and genetic factors but also by external environmental factors that accelerate aging, such as UV radiation.[5,6]

There are differences in clinical signs between natural skin aging and photoaging. Natural skin aging is marked by weakness and fine wrinkles but is not related to increased pigmentation, deep wrinkles, or rough appearance, which are hallmarks of skin photoaging. The differences between natural skin aging and photoaging also involve histological aspects.[7] In skin with natural aging, epidermal atrophy is observed along the flattening of dermal-epidermal border and thin dermis. In contrast, skin with early aging is often related to epidermal thickening and severe disruption of dermal connective tissue, including accumulation of elastin-contained substances, which is recognized as solar elastosis.[8] This condition triggers increased formation of wrinkles and progressive color change due to cumulative alteration regarding the skin's structural, physiological, and appearance layers, particularly those exposed to the sunlight.[8-11]

Preventive and therapeutic strategies for skin aging have used retinoic acid, alpha hydroxyl acid, antioxidants, estrogens, and growth factors.[12] Currently, agents of the community's choice come from natural sources due to relatively low toxicity in clinical application.[13] One of the herbal sources with phenolic, terpenoids, anthocyanin, tannin, and flavonoid contents is the butterfly pea flower (*Clitoria Ternatea L*).[14]

This study aimed to analyze the effect of a nanoemulsion spray containing butterfly pea flower (*Clitoria ternatea L*) on signs of skin aging due to exposure to UV.

METHODS

This experimental study used allocation randomization sampling to determine the experiment and control group. The making of ethanol extract from butterfly pea flower and intervention in aging model rats were completed in the Pharmacy Laboratory of Universitas Islam Bandung. Making of nanoparticles was completed in the Pharmacy Laboratory of Universitas Islam Indonesia. At the same time, Verhoeff staining of rat skin tissue was done in the Histology Laboratory of Universitas Gadjah Mada from January- July 2023. This study has been granted a permit by Komite Etik Penelitian Kesehatan Universitas Islam Bandung No.092/KEPK-UNISBA/2023.

Maceration and extraction methods of butterfly pea flower were commenced based on previous studies with minor modifications.

e-ISSN 2774-2318
p-ISSN 2407-0505

Fresh butterfly pea flower (*Clitoria ternatea L*), 1 kilogram, was macerated using 5 liters of ethanol 95% for three days, then evaporated by a rotary evaporator to acquire viscous extract. The making process of butterfly pea flower extract nanoemulsion started with mixing labral contents (Gattefose, Prancis), tween 80, and Propylene Glycol (Bratachem, Indonesia) with 2:2:1 ratio as primary emulsion, proceeded with mixing by ultrasonic probe (Biologic,150VT, USA) by frequency of 20 kHz with 40 % amplitude strength, 60% pulser for 2 minutes in ice bath. After, 1 % or 3% extracts of butterfly pea flower were added to the primary emulsion mixture, further mixed by an ultrasonic probe (Biologic,150VT, USA) by 20 kHz frequency with 40% amplitude strength, 60 % pulser for 2 minutes in an ice bath. 100 mL of aquadest was added before mixing with a magnetic stirrer for 10 minutes. The result was subjected to nanoparticle measurement using Particle Size Analyzer (PSA) and then moved to a spray bottle for further research.

Animal Model

Twenty-four healthy male Wistar Rat with 250 ± 25 g CV=10% weight approximation were fed ad libitum and housed at 28ºC temperature with a 12-hour photoperiod. After one week of acclimatization, rats were assigned by randomization into three groups: positive control, UVB irradiation control (negative control); UVB irradiation and nanoemulsion spray of *Clitoria ternatea* extract (NESCT) 1% once daily; UVB irradiation and (NESCT) 1% twice daily; UVB irradiation and (NESCT) 3% once daily; UVB irradiation and (NESCT) 3% twice daily. Each group consisted of four rats. This study utilized a UVB irradiation lamp (G25T8E, Sankyo Denki Co., Kanagawa, Japan) with peak emission at 306 nm. with peak emission at 302 nm CL-100M, UVP, USA). Rats with shaved fur at the back area were exposed to 160 mJ/cm^2 UVB light for 30 minutes for 14 consecutive days, according to previous studies with minor modifications. Spray was administered daily and topically to the back area of the skin until day 14. Control rats did not receive any medication. On day 15, all rats were terminated to isolate the skin tissue.

Verhoff Staining

1 cm x 1 cm skin tissues were taken and fixed in 10% formaldehyde for 24 h, then embedded in paraffin and sectioned (6 µm). The sections were stained with Verhoeff–Van Gieson to visualize collagen and elastin production. Interpretation was carried out by two experts (anatomical pathology and histology). Extracellular matrix degradation is measured by observing the percentage of skin extracellular matrix tissue damage.

Collagen and Elastin Score

Distribution of elastin: positive 1: 1 from 4 areas; positive 2: 2 of 4 areas; positive 3: >2 of 4 **region**s. Collagen of distribution: positive 1: < 20%; positive 2: ≥ 20-50%; positive 3: >50-80%; positive 4: >80%. Collagen intensity: Positive 1: collagen fibers stained with weak intensity; positive 2: moderate intensity staining of collagen fibers; positive 3: collagen fibers are stained with s**olid** intensity. Collagen and Elastin Score: Distribution of elastin + Collagen of distribution + Collagen intensity

Statistical analy**s**es were performed using the software GraphPad Prism. All data are presented as mean ± standard deviation (SD).

e-ISSN 2774-2318
p-ISSN 2407-0505

The data obtained were collected, compiled, and tested for normality with the Shapiro-Wilk test and the homogeneity test with the Lavene test. Data analysis used one-way ANOVA and continued with Tukey's post hoc test using p-value <0.05.

RESULTS

Comparison of the epidermis and extracellular matrix in each treatment group using Verhoeff staining (Fig 1 and 2). Figure 1 shows the positive control group (PC) at 100x magnification showed a normal, evenly thin epidermis layer (black arrow), the dermis layer was filled with extracellular connective tissue matrix, there were no areas of degradation, and vigorous collagen intensity was visible throughout—area (green arrow). Meanwhile, in the negative control CN, the epidermis layer thickened in several regions, reacting to UV exposure. In the dermis layer, heavy degradation of the extracellular matrix appeared. The group given NESCT (D2, D3, and D4) showed a thin epidermis layer (black arrow), the same as the normal group, indicating no damage to the epidermis. The dermis layer shows mild extracellular degradation with vigorous collagen intensity, which suggests mild damage to the dermis layer and repair has occurred during the process. New collagen deposition in most areas.

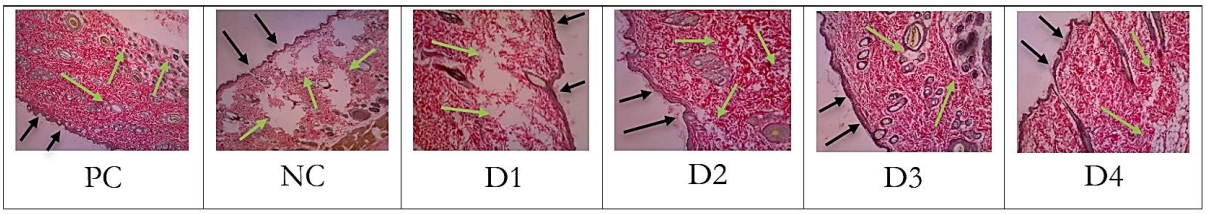

Figure 1. Description of epidermis and extracellular matrix. The black arrow is the epidermis, and the green arrow is the extracellular matrix; Verhoeff staining magnification is 100x.

PC: positive control, NC: UVB irradiation control (negative control), D1: UVB irradiation and NESCT 1% once daily, D2: UVB irradiation and (NESCT) 1% twice daily, D3: UVB irradiation and (NESCT) 3% once daily, D4: UVB irradiation and (NESCT) 3% twice daily.

Comparison of collagen and elastin in each treatment group using Verhoeff staining

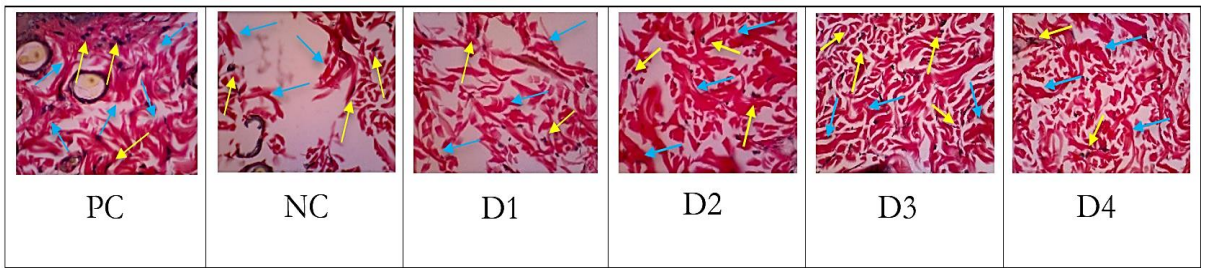

Figure 2. Description of collagen and elastin: The blue arrow is collagen, and the yellow arrow is elastin; Verhoeff staining magnification is 400x.

PC: positive control, NC: UVB irradiation control (negative control), D1: UVB irradiation and NESCT 1% once daily, D2: UVB irradiation and (NESCT) 1% twice daily, D3: UVB irradiation and (NESCT) 3% once daily, D4: UVB irradiation and (NESCT) 3% twice daily.

e-ISSN 2774-2318
p-ISSN 2407-0505

Figure 2 shows the positive control group at 400x magnification. The dermis layer is filled with extracellular connective tissue matrix, collagen (blue arrow), and elastin (yellow arrow). There are no areas of degradation, and vigorous collagen intensity appears throughout the region (green arrow). In the negative control group, the dermis layer showed very few thin elastin (yellow arrow) and collagen fibers (blue arrow), with a localized distribution in only one of the quadrants. This result indicates damage to elastin and collagen fibers due to UV radiation. The group given NESCT (D2, D3, and D4) showed in the dermis layer, there are many thick elastin fibers (yellow arrow) and (yellow arrow) with an even distribution in all quadrants, which indicates that there is no damage to the elastin fibers in the dermis layer.

Effect of nanoemulsion spray of *Clitoria ternatea* extract (NESCT) on extracellular matrix degradation

The effect of *Clitoria ternatea* extract nanoemulsion spray (NESCT) **on** extracellular matrix degradation of rat skin dermis tissue is presented in Figure 3. The normality analysis test was carried out using the Shapiro-Wilk goodness-of-fit test, and the test results with α=5% showed that at a confidence level of 95%, all data were normally distributed (p>0.05). Figure 3 shows the presentation of extracellular matrix degradation (%) in the positive control group (4,375± 1.25;) negative control group (75 ± 5,77); Treatment group D1 (46,25 ± 2,5); Treatment group D2 (13,75 ± 2,5); treatment group D3 (10±4,082); treatment group D4 (7,5 ± 2,89). Tukey's post hoc analysis displays significant differences regarding extracellular matrix degradation between the negative control group and the positive control group, as well as between intervention groups D1, D2, D3, and D4. Other than those results, there is no significant difference between **the** positive control and intervention groups D1, D, D3, and D4.

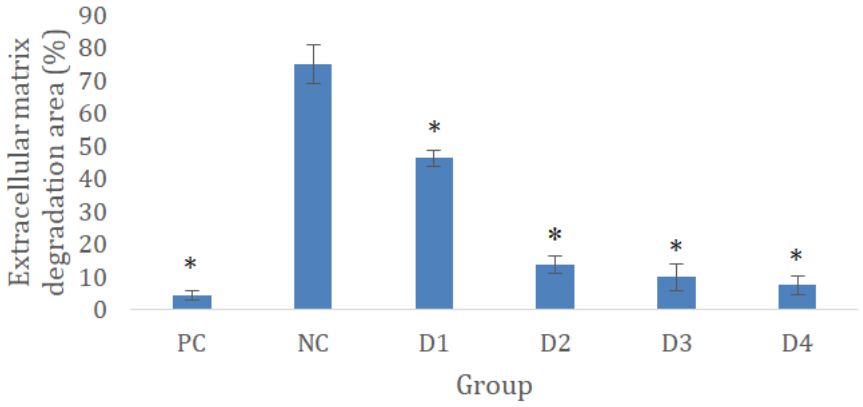

Figure 3. Effect of NESCT on extracellular matrix degradation, * (Tukey's post hoc test p-value <0.05 compared to NC)

PC: positive control, NC: UVB irradiation control (negative control), D1: UVB irradiation and NESCT 1% once daily, D2: UVB irradiation and (NESCT) 1% twice daily, D3: UVB irradiation and (NESCT) 3% once daily, D4: UVB irradiation and (NESCT) 3% twice daily.

e-ISSN 2774-2318
p-ISSN 2407-0505

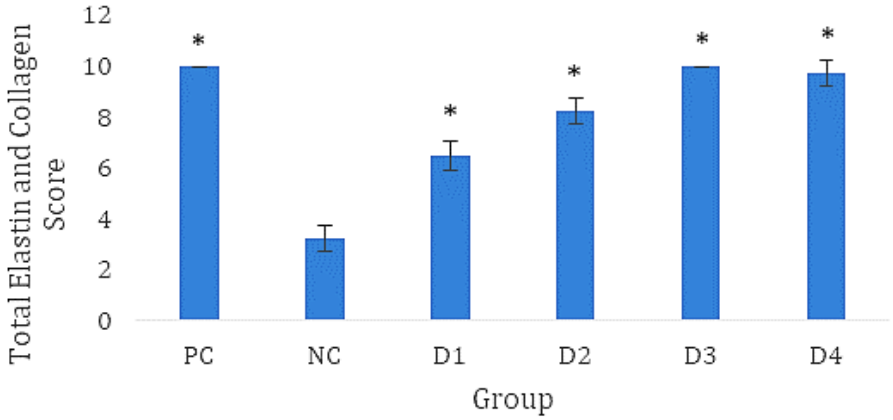

Figure 4. The Effect of *Clitoria ternatea* extract nanoemulsion spray (NESCT) on the Total Elastin and Collagen Score, * (Tukey's post hoc test p-value <0.05 compared to NC)

PC: positive control, NC: UVB irradiation control (negative control), D1: UVB irradiation and NESCT 1% once daily, D2: UVB irradiation and (NESCT) 1% twice daily, D3: UVB irradiation and (NESCT) 3% once daily, D4: UVB irradiation and (NESCT) 3% twice daily.

The effect of *Clitoria ternatea* Extract Nanoemulsion Spray (NESCT) on the Total Elastin and Collagen Score

The effect of *Clitoria ternatea* extract nanoemulsion spray (NESCT) **on the** Total Elastin and Collagen Score of rat skin dermis tissue is presented in Figure 4. The normality analysis test was carried out using the Shapiro-Wilk goodness-of-fit test, and the test results with α = 5% showed that at a confidence level of 95%, all data were normally distributed (p>0.05). Figure 1 shows the presentation of extracellular matrix degradation (%) in the positive control group (10,00± 0;) negative control group (3,25± 0,5)**,** Treatment group D1 (6,5 ± 0,58); Treatment group D2 (8,25 ± 0,5); treatment group D3 (10± 0); treatment group D4 (9,75 ± 0,5). Tukey post hoc analysis found significant differences in total elastin and collagen scores between **the** negative **and positive control groups**. Likewise, there is **a** significant difference between **the** control and intervention groups D1, D2, D3, and D4. On the other hand, there is no significant difference between **the** positive control group and intervention groups D1, D, D3, and D4.

DISCUSSION

Skin aging occurs due to reduced antioxidants; a single UV exposure may decrease antioxidants to almost half the amount.[15] Histologically, aging was marked by epidermal disruption, a decline in fibroblast and collagen fibers amount, thin dermis, and inappropriate functions.[16] These conditions may stem from subcutaneous keratinocyte dysfunction, declined stem cell regenerative capabilities in the epidermal basal layer, and decreased skin restorative function, leading to aging.[17] Secondly, due to aging-related accumulation of skin damage and dysfunction, fibroblasts may lose the ability to reform extracellular matrix or have reduced function regarding collagen or viscous protein synthesis or secretion. Thirdly, fibroblast aging may alter intracellular homeostasis through a specific paracrine mechanism.[18,19]

Photo aging with UVB irradiation may increase matrix metalloproteinase (MMP) and induce collagen matrix degradation in the

e-ISSN 2774-2318
p-ISSN 2407-0505

dermal layer of the skin. Also, a UV-induced increase in reactive oxygen species (ROS) may influence protein kinase phosphorylation in the activated MAPK signaling pathway.[20] UV exposure also may activate MAPK signaling and increase the activity of activator protein-1 (AP-1) and expression of MMP that degrades extracellular matrix (ECM) protein, such as collagen, which contributes to tensile strength in the dermis.[21] Other than those conditions, inflammation factors are activated by ROS, decreasing the abundance of ECM protein by increasing MMP or cyclooxygenase-2 and simultaneously reducing collagen, pro-collagen precursor amount.[22,23]

Butterfly pea flower contains tannins, phlorotannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, Flavonol glycosides, proteins, alkaloids, antharaquinone, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils, and steroids.[17,24,25] Petals of butterfly pea flower are light blue, consisting of various polyphenols, particularly flavonoid anthocyanin. The main polyphenol content within CT petals is anthocyanin ternatin.[17] Polyphenol is a secondary metabolite in herbs found abundantly in tea, curcumin, and fruits and is frequently utilized as an anti-aging agent. Polyphenol reduces skin oxidative damage and inflammation through antioxidant and anti-inflammatory effects, especially by inhibiting collagen degradation, increasing collagen synthesis, and reducing inflammation, which involves regulation of MMP, cytokines, and signaling pathways such as Nrf2, NF-ϰB, and MAPK.[26,27]Also, tannin contents in butterfly pea flower can induce collagen formation. These results support previous studies that state gallotannin (tannin) can induce collagen synthesis and inhibit MMP-1. Tannin also has antioxidant properties, increasing glutathione concentration. Thus, gallotannin may potentially be a novel anti-wrinkle agent.[28]

CONCLUSION

Spray nanoemulsion preparations containing butterfly pea flower extract (*Clitoria ternatea* L) have antiaging activity by inducing epidermis repair formation of extracellular matrix, collagen, and elastin. Due to each of the four concentrations of nanoemulsion spray preparation of ethanol extract of butterfly pea flower (*Clitoria ternatea L*) (NESCT) effect of increasing collagen and elastin growth as well as inhibiting extracellular matrix degradation, we recommend using 1% concentration once daily.

REFERENCES

1. Rattanawiwatpong P, Wanitphakdeedecha R, Bumrungpert A, Maiprasert MJJocd. Antiaging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: a split-face, randomized controlled trial. 2020;19(3):671-6.
2. Orioli D, Dellambra EJC. Epigenetic regulation of skin cells in natural aging and premature aging diseases. 2018;7(12):268.
3. Fuchs EJCtidb. Epithelial skin biology: three decades of developmental biology, a hundred questions answered and a thousand new ones to address. 2016;116:357-74.
4. Luiten KAJARR. Oxidation events and skin aging. 2015;21:16-29.
5. Gu Y, Han J, Jiang C, Zhang YJArr. Biomarkers, oxidative stress and autophagy in skin aging. 2020;59:101036.

e-ISSN 2774-2318
p-ISSN 2407-0505

6. Koohgoli R, Hudson L, Naidoo K, Wilkinson S, Chavan B, Birch-Machin MAJEd. Bad air gets under your skin. 2017;26(5):384-7.
7. Quan T, Fisher GJJG. Role of age-associated alterations of the dermal extracellular matrix microenvironment in human skin aging: a mini-review. 2015;61(5):427-34.
8. Poon F, Kang S, Chien ALJP, photoimmunology, photomedicine. Mechanisms and treatments of photoaging. 2015;31(2):65-74.
9. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CCJD-e. Skin antiaging strategies. 2012;4(3):308-19.
10. Zingare ML, Zingare PL, Dubey AK, Ansari MAJIJoP, Sciences B. Clitoria ternatea (Aparajita): a review of the antioxidant, antidiabetic and hepatoprotective potentials. 2013;3(1):203-13.
11. Montenegro L, Sinico C, Castangia I, Carbone C, Puglisi GJIjop. Idebenone-loaded solid lipid nanoparticles for drug delivery to the skin: In vitro evaluation. 2012;434(1-2):169-74.
12. Ramos-e-Silva M, Celem LR, Ramos-e-Silva S, Fucci-da-Costa APJCid. Antiaging cosmetics: Facts and controversies. 2013;31(6):750-8.
13. Kim J, Cho SY, Kim SH, Cho D, Kim S, Park C-W, et al. Effects of Korean ginseng berry on skin antipigmentation and antiaging via FoxO3a activation. Journal of ginseng research. 2017;41(3):277-83.
14. Zakaria NNAB. Evaluation of anti-ageing properties of Moringa oliefera Lam., Centella asiatica (L.) Urban, Clitoria ternatea L. and Cosmos caudatus Kunth. for potential application as cosmeceuticals: Newcastle University; 2019.
15. Abla M, Banga AJIjocs. Formulation of tocopherol nanocarriers and in vitro delivery into human skin. 2014;36(3):239-46.
16. Karim PL, Aryani IA. Anatomy and histologic of intrinsic aging skin. Bioscientia Medicina: Journal of Biomedicine and Translational Research. 2021;5(11):1065-77.
17. Panich U, Sittithumcharee G, Rathviboon N, Jirawatnotai S. Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. Stem cells international. 2016;2016.
18. Tobin DJ. Introduction to skin aging. Journal of tissue viability. 2017;26(1):37-46.
19. de Araújo R, Lôbo M, Trindade K, Silva DF, Pereira N. Fibroblast growth factors: a controlling mechanism of skin aging. Skin pharmacology and physiology. 2019;32(5):275-82.
20. Muthusamy V, Piva TJ. The UV response of the skin: a review of the MAPK, NFκB and TNFα signal transduction pathways. Archives of dermatological research. 2010;302:5-17.
21. Quan T, Little E, Quan H, Voorhees JJ, Fisher GJ. Elevated matrix metalloproteinases and collagen fragmentation in photodamaged human skin: impact of altered extracellular matrix microenvironment on dermal fibroblast function. The Journal of investigative dermatology. 2013;133(5): 1362.
22. Petruk G, Del Giudice R, Rigano MM, Monti DM. Antioxidants from plants protect against skin photoaging. Oxidative medicine and cellular longevity. 2018;2018.
23. Fu Y, Li C, Wang Q, Gao R, Cai X, Wang S, et al. The protective effect of collagen peptides from bigeye tuna (Thunnus obesus) skin and bone to attenuate UVB-induced photoaging via MAPK and TGF-β signaling pathways. Journal of Functional Foods. 2022;93:105101.
24. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine Clitoria ternatea—From traditional use to

e-ISSN 2774-2318
p-ISSN 2407-0505

scientific assessment. Journal of ethnopharmacology. 2008;120(3):291-301.
25. Rai SS, Atanu B, Ankit S, Meenu S. Evaluation of anti-ulcer activity of aqueous and ethanolic extract of whole plant of Clitoria ternatea in albino wistar rats. International Journal of Pharmaceutical Sciences and Drug Research. 2015;7(1):33-9.
26. Davinelli S, Bertoglio JC, Polimeni A, Scapagnini GJCPD. Cytoprotective polyphenols against chronological skin aging and cutaneous photodamage. 2018;24(2):99-105.
27. Chuang S-Y, Lin Y-K, Lin C-F, Wang P-W, Chen E-L, Fang J-YJN. Elucidating the skin delivery of aglycone and glycoside flavonoids: How the structures affect cutaneous absorption. 2017;9(12):1304.
28. Ryeom GGM, Bang WJ, Kim YB, Lee GE. Gallotannin improves the photoaged-related proteins by extracellular signal-regulated kinases/c-Jun N-terminal kinases signaling pathway in human epidermal keratinocyte cells. Journal of medicinal food. 2018;21(8):785-92.