turnitin

Correction Manuscript - TNJPR - 1.docx
Mar 11, 2022
4240 words / 24556 characters

Verawati Verawati

Correction Manuscript - TNJPR - 1.docx

Sources Overview

13%

OVERALL SIMILARITY

1	bidd2.nus.edu.sg INTERNET	1%
2	www.science.gov INTERNET	<1%
3	www.hindawi.com INTERNET	<1%
4	tjpr.org INTERNET	<1%
5	www.mdpi.com INTERNET	<1%
6	allie.dbcls.jp INTERNET	<1%
7	www.lookchem.com INTERNET	<1%
8	Mostefa Lefahal, Nabila Zaabat, Radia Ayad, El Makhloufi et al. "In Vitro Assessment of Total Phenolic and Flavonoid Contents, Antioxi... CROSSREF	<1%
9	Fatemeh Yousefbeyk, Ghazaleh Hemmati, Ziba Gholipour, Saeed Ghasemi et al. " Phytochemical analysis, antioxidant, cytotoxic, and an... CROSSREF	<1%
10	Shahrzad Aslany, Farzaneh Tafvizi, Vahid Naseh. "Characterization and evaluation of cytotoxic and apoptotic effects of green synthesi... CROSSREF	<1%
11	www.ncbi.nlm.nih.gov INTERNET	<1%
12	journaljpri.com INTERNET	<1%
13	K Kartini, N I E Jayani, N D Octaviyanti, A H Krisnawan, C Avanti. "Standardization of Some Indonesian Medicinal Plants Used in "Scienti... CROSSREF	<1%
14	innovareacademics.in INTERNET	<1%
15	www.researchsquare.com INTERNET	<1%
16	Preprint source Chae Yeon Hwang, Yuniwaty Halim, Marcelia Sugata, Dela Rosa, Sherlyn Putri Wijaya, Eden Steven. "Assessment of Agaricus bisporus ... CROSSREF POSTED CONTENT	<1%

17	Dingfa Wang, Luli Zhou, Hanlin Zhou, Guanyu Hou. "Chemical composition and anti-inflammatory activity of n-butanol extract of Piper s... CROSSREF	<1%
18	vdocuments.site INTERNET	<1%
19	Hu, Ting, Xiao-Wei He, and Jian-Guo Jiang. "Functional Analyses on Antioxidant, Anti-inflammatory, and Antiproliferative Effects of Extr... CROSSREF	<1%
20	P H Riyadi, W A Tanod, D Wahyudi, E Susanto, A S Fahmi, S Aisiah. "Potential of tilapia (Oreochromis niloticus) viscera bioactive peptid... CROSSREF	<1%
21	Sang-Won Lee, Jeong Min Seo, Min-Ki Lee, Jin-Hyuk Chun et al. "Influence of different LED lamps on the production of phenolic compo... CROSSREF	<1%
22	repository.uhamka.ac.id INTERNET	<1%
23	Gervason Moriasi, Anthony Ireri, Mathew Piero Ngugi. " In Vitro Antioxidant Activities of the Aqueous and Methanolic Stem Bark Extrac... CROSSREF	<1%
24	Khaing zar Myint, Ke Wu, Yongmei Xia, Ye Fan, Jie Shen, Peter Zhang, Jianxin Gu. " Polyphenols from (Bertoni) leaves and their function... CROSSREF	<1%
25	Kunal Bhattacharya, Ripunjoy Bordoloi, Nongmaithem Randhoni Chanu, Ramen Kalita, Bhargab Jyoti Sahariah, Atanu Bhattacharjee. "In... CROSSREF	<1%
26	www.proderm.de INTERNET	<1%
27	A. N. Assimopoulou, Z. Sinakos, V. P. Papageorgiou. "Radical scavenging activity ofCrocus sativus L. extract and its bioactive constitue... CROSSREF	<1%
28	Duy Quang Dao, Thi Thu Trang Phan, Thi Le Anh Nguyen, Phan Thi Hoai Trinh et al. "Insight into Antioxidant and Photoprotective Prope... CROSSREF	<1%
29	Francislene J. Martins, César A. Caneschi, José L.F. Vieira, Wagner Barbosa, Nádia R.B. Raposo. "Antioxidant activity and potential phot... CROSSREF	<1%
30	eprints.unm.ac.id INTERNET	<1%
31	japsonline.com INTERNET	<1%
32	oamjms.eu INTERNET	<1%
33	phytohub.eu INTERNET	<1%
34	res.mdpi.com INTERNET	<1%
35	www.jyoungpharm.org INTERNET	<1%
36	A.G.A.W. Alakolanga, A.M.D.A. Siriwardane, N. Savitri Kumar, Lalith Jayasinghe, Rakesh Jaiswal, Nikolai Kuhnert. "LC-MSn identification... CROSSREF	<1%
37	"Abstracts of the Asian Congress of Nutrition 2019", Annals of Nutrition and Metabolism, 2019 CROSSREF	<1%
38	"Pharmacotherapeutic Botanicals for Cancer Chemoprevention", Springer Science and Business Media LLC, 2020 CROSSREF	<1%
39	"Sunlight, Vitamin D and Skin Cancer", Springer Science and Business Media LLC, 2020 CROSSREF	<1%

40 Annabelle St-Pierre, Dorian Blondeau, Michelle Boivin, Vickie Beaupré, Nathalie Boucher, Isabel Desgagné-Penix. "Study of antioxidant p… <1%
CROSSREF

41 Komar Ruslan, Shelvy Happyniar, Irda Fidrianny. "Antioxidant potential of two varieties of Sesamum indicum L. collected from Indonesi… <1%
CROSSREF

42 Naing Naylin, Toshihiko Suganuma, Ok Taing. " Antioxidant Activities of Compounds Isolated from Fermented Broth of ", Food Biotechn… <1%
CROSSREF

Excluded search repositories:

None

Excluded from document:

Bibliography

Excluded sources:

None

POTENTIAL PHOTOPROTECTIVE EFFECT OF POLAR EXTRACT OF *Elephantopus mollis* THROUGH THE INVITRO AND INVIVO EVALUATION APPROACHES

Verawati[1,4]* , Almahdy A.[2], Febriyenti[3], Deddi Prima Putra[1]

[1]Department of Biology Pharmacy, Faculty of Pharmacy, Andalas University, West Sumatera-25175, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Andalas University, West Sumatera-25175, Indonesia
[3]Department of Pharmaceutics, Faculty of Pharmacy, Andalas University, West Sumatera-25175, Indonesia
[4]Faculty of Pharmacy, Universitas Perintis Indonesia, West Sumatera-25586, Indonesia

*Corresponding author : Tel. +6281374094606 ; Email : verawati@upertis.ac.id ; 1931012001_verawati@student.unand.ac.id

ABSTRACT

Exposure to ultraviolet radiation from the sun can cause various harmful effects such as erythema, wrinkles, dark spots, sunburn that trigger premature aging to skin cancer. This research aimed to explore the photoprotective potential of the nonpolar, semipolar, and polar extracts of *Elephantopus mollis* herbs through invitro and invivo evaluation. Invitro evaluation was done by examining antioxidant activity, determination of sun protection factor value, total phenolic content, and identification of the chemical components of the extracts. The most active extract on invitro evaluation continued invivo testing with observation parameters of erythema and wrinkle formation and dermal histopathology of mice exposed to radiation from Ultraviolet lamps. The invitro examination showed the polar extract had the best photoprotective potential compared to other extracts, with intense antioxidant activity (IC_{50} 46.55 µg/ml), total phenolic levels of 109.2 mg/g extract GAE (gallic acid equivalent) and, SPF values of 13.5. Topical administration of polar extract with increased doses showed improvement in skin performance and dermis of mice exposed to ultraviolet radiation. Polar extracts can significantly reduce erythema, wrinkles, and dermis histological performance similar to the typical skin structure of mice. Based on LC-HRMS analysis, the polar extract contains bioactive components of polyphenols and flavonoids such as caffeic acid, chlorogenic acid, rutin, trifolin, and 3,5-di-O-caffeoylquinic acid. Polar extract of *Elephantopus mollis* can be developed as a raw material for drugs or cosmetics that serve as photoprotector and antiphotoaging and to repair skin damage due to ultraviolet rays.

Keywords: *Elephantopus mollis*, ultraviolet radiation, antioxidant, phenolic, photoprotective

INTRODUCTION

Exposure to solar ultraviolet radiation can promote the formation of radical species in the skin and evoke an oxidative stress state responsible for various damage to the skin. [1,2] Skin exposed to repeated sunlight may suffer from erythema, wrinkles, inflammation, and sunburn resulting in premature aging and skin cancer.[3,4] The adverse effects of sunlight can be overcome through prevention and treatment using products that work with a combination of sunscreen, antioxidants, and agents to repair skin damage.[5,6] Skin protection agents against

the adverse effects of sunlight can be sourced from synthetic and natural compounds consisting of organic or inorganic substances.[7]

Various commercial sunscreen and antiaging products contain many synthetic ingredients and have several harmful effects.[8] For example, synthetic substances such as benzophenone, octocrylene, and their derivatives are potentially carcinogenic.[8,9] The use of synthetic materials over a long period can cause allergies, pain in the skin, acne, and even tumors or skin cancer.[10] Health-harmful threats from synthetic products provide opportunities for the use of natural ingredients such as plant extracts that contain bioactive substances[5,11,12]. Plants from the family Asteraceae are known to be rich in phenolic components that are efficacious as organic sunscreens and active antioxidants.[13,14] One of the Asteraceae plants commonly found in the province of West Sumatra, Indonesia, is *Elephantopus mollis*.

E. mollis has been reported to have anti-inflammatory, anti-tyrosinase, antimelanogenesis, and anticancer properties.[15,16] This research aimed to explore the photoprotection potential of *Elephantopus mollis* on the skin in counteracting the adverse effects of repeated UV exposure. The bioactivity evaluation approach of *E. mollis* is comprehensively conducted using in vitro and in vivo examination. This study exposes the opportunity to develop *E. mollis* as a raw material for medicinal and cosmetic products that can overcome skin problems due to UV exposure, especially in Indonesia, a tropical country with sun exposure throughout the year.

EXPERIMENTAL

Materials and Methods

Chemical and Reagen

Gallic acid, DPPH, sodium carbonate, reagent Folin Ciocalteu were purchased from Merck, n-hexane, ethyl acetate, methanol and aquadest are obtained from suppliers of chemicals in Indonesia such as Mutiara Lab Sains, Nitra Kimia and Bratachem.

Plant Material

Elephantopus mollis plant taken in August 2020 in Pariaman City of West Sumatra Province, Indonesia. This plant was identified in the Herbarium Biology University of Andalas (ANDA). The aerial part of the plant was used in this study.

Procedure Extraction of *Elephantopus mollis*

The dried powder of the aerial part of *E. mollis* was extracted by the step gradient polarity of maceration method that started using n-hexane as the first extraction solvent. The plant residue remaining from the filtration of n-hexane was macerated using ethyl acetate as the second solvent. Furthermore, the residue of ethyl acetate was soaked using the third solvent, methanol. Each maceration step was done for 48 hours with triplicate for every type of solvent. The filtrate from each extracting solvent was concentrated using a rotary evaporator to obtain the viscous extract, a nonpolar extract from hexane solvent, semipolar extract from ethyl acetate solvent, and polar extract from methanol solvent.

Characterization of extracts

The three types of *E. mollis* extract were characterized by observing organoleptic, phytochemical screening, percentage of yield, loss on drying, and total ash content using the methods listed in the Indonesian Herbal Pharmacopoeia.[17]

InVitro Evaluation

Determination of Phenolic Content

Total phenolic content was determined by the Folin Ciocalteu method and used gallic acid as a standard compound.[18] 0.5 ml of each extract (1000 µg/ml) was mixed with a 5 ml Folin -Ciocalteu (10%) reagent and 4 ml of sodium carbonate solution (7.5%). The mixtures were incubated at room temperature for 15 minutes. The absorbance of test solutions was measured using a spectrophotometer at a wavelength of 762 nm. Gallic acid as a standard solution was prepared in 20, 40, 60, 80, and 100 µg/ml concentration series.

Examination of Antioxidant Activity

The antioxidant activity of *Elephantopus mollis* extracts was measured by the radical DPPH (2,2-diphenyl-1-picrilhydrazine) scavenging method.[19] The test solutions of extracts were made in various series of concentrations, then pipetted 2 ml and mixed with 4 ml of DPPH solution 50 µg/ml. The control solution using 4 ml of DPPH 50 µg/ml was mixed with 2 ml of methanol. Gallic acid was used as an antioxidant standard compound and methanol as a blank solvent. All test solutions (extracts, standard, and control) were incubated for 30 minutes in the dark. Then, the absorbance of solutions was measured at a wavelength of 516 nm. Antioxidant activity was expressed as % inhibition and IC_{50} (concentration of compounds that inhibit 50% of DPPH radicals).

Determination of Sun Protection Factor (SPF)

Sun Protection Factor (SPF) values were determined using a spectrophotometer by measuring the absorbance of extract solutions in a cuvette with the thickness of 1 cm.[20] Nonpolar, semipolar and polar extract of *Elephantopus mollis* were prepared in concentrations of 100 µg/ml and 250 µg/ml. The absorbance of each extract solution was examined at a wavelength range of 290 nm – 320 nm at intervals of 5 nm. The value of absorbances was incorporated into Sayre's mathematical equation.

InVivo Evaluation

Treatment of Test Animals

In vivo testing using animals was continued on *E. mollis* extract, which has the best activity based on the results of the invitro evaluation. White male mice acclimatized for seven days were divided into five groups consisting of 6 mice, the normal control group, the UV control group, and extract groups. The normal control group consisted of normal or non-radiated mice, while the UV control was the group of mice that were given UV exposure. The extract groups were mice exposed to UV rays and were treated with *E mollis* active extract by using a topical application on the shaved dorsal of mice at the concentration of 2.5 %; 5%, and 7.5% as much as 200 mg per application. Topical administration of each dose of *E. mollis* extract was done daily for 14 days.

Sources of Ultraviolet radiation using 13 Watt Reptile UV lamps (Exo Terra®). The distance of the lamp to the mice was 20 cm to obtain 300 µW/cm^2 of UV radiation intensity.[21] UV control and extract groups were irradiated for 2 hours, three times a week for two weeks. The mice received energy at 2.16 J/cm^2 for each radiation exposure[21]. Everyday observations were made for erythema and wrinkles on the dorsal area. On the 15th day, the mice were euthanized, the dorsal region was skinned, and the skin tissue was prepared for a histopathological examination with Hematoxylin-Eosin staining.

Examination of chemical components with Liquid Chromatography – High Resolution Mass Spectrometry (LC – HRMS)

The chemical components of the active extract *E. mollis* were examined with non-targeted screening by using LC – HRMS instrument. The instrument used for separation was HPLC Thermo Scientific Dionex Ultimate 3000 RSLC nano with a micro flow meter. Instrumen used for Mass Spectroscopy was High Resolution Mass Spectrometer: Thermo

Scientific Q Exactive by using Processing data software: Compound Discoverer with mzCloud MS/MS Library

RESULTS AND DISCUSSION

Elephantopus mollis herb was extracted using a step gradient polarity of extracting solvent, that aimed to group compounds in plant based on their polarity. The extraction procedure chosen was maceration because it is simpler and can protect the thermostable compounds. The results of the characterization of 3 types of *E. mollis* extract can be seen in table 1. The appearance of organoleptic of all three kinds of extracts looks different and so its physical character. Polar extract provides higher in percentage of yield, loss on drying, and total ash content compared to semipolar and nonpolar extracts. In the observation of phytochemical screening, the polar extract is also rich in phytochemical groups compared to 2 other types of extracts.

Antioxidant activity and total phenolic content of *E. mollis* extracts are shown in table 2. The result of each extract is remarkably different, and the polar extract indicates the most potential value. Antioxidant activity was tested using the radical method DPPH with the principle of the reaction mechanism is the electron donor of antioxidant substances to DPPH radicals to complete and stabilize DPPH electrons. Visually, antioxidant activity can be observed by discoloring DPPH solutions that are initially dark purple to yellowish after reacting with antioxidant substances. Antioxidant activity is indicated by the value of IC_{50}, which is the concentration of antioxidant substances that can scavenge DPPH radicals by 50%. The lower the IC_{50} value indicates more potent antioxidant activity. The antioxidant activity of each extract is different, and the polar extracts showed excellent antioxidant activity with IC_{50} lower than other extracts. The antioxidant activity of polar extract can be categorized very strongly (< 50 ppm)) while semipolar extracts are moderate activity (101 - 250 ppm) and nonpolar extracts are weak (250 - 500 ppm).[22]

The highest total phenolic levels obtained in polar extracts were 109.2 mg/g GAE, higher than semipolar and nonpolar extracts. Examination of total phenolic content using the Folin Ciocalteu (FC) method based on a redox reaction mechanism between phenolic substances that reduce the acidic components in the reagent (FC) in an alkaline atmosphere. Phenolic substances turn into phenolic ions, while the acidic components of reduced FC reagents will result in discoloration from yellow to deep blue. This discoloration is stoichiometrically proportional to the number of phenolic substances that react.[23]

Pearson's correlation analysis showed a correlation between antioxidant activity and total phenolic levels, amounting to -0.864. This figure shows a perfect correlation, while the negative value is due to an inversely proportional relationship, where the higher the total phenolic level, the lower the IC_{50} value of antioxidants. The group of phenolic compounds is a natural antioxidant whose potency is due to its ability to donate hydrogen atoms to radical compounds. In contrast, the phenolic radicals formed will stabilize through the delocalization of electrons in aromatic ring resonance. The antioxidant activity of the phenolic will be more potent with the presence of two hydroxyl groups close together at the ortho position. The presence of other substituents in the structure of phenolic compounds and the bond strength of the O-H group also affect its antioxidant activity.[24]

SPF values were measured at UVB wavelengths. In the range of 290 nm – 320 nm. Polar extract at a concentration of 250 ppm gives an SPF value of 13.85, the highest compared to other extracts. SPF values of each extract and absorbant measurements at intervals of 5 nm in the UVB region are shown in table 3.

The SPF ability of polar extract belongs to the category of Maximum Protection. UV radiation can cause acute effects such as erythema, pigmentation, and sunburn. The chronic impact of this radiation is susceptible to individuals who work daily in open areas and are

exposed to direct sunlight to suffer the effects of immune system suppression, inflammation, and photoaging.[25] Polar extract *E. mollis* has a high phenolic content and vigorous antioxidant activity and can also act as an organic sunscreen that will reduce the penetration of UV rays into the skin.

Organic substances such as phenolic components in plants can serve as sunscreens because they have chromophore groups that absorb sunlight energy.[26] The energy of sunlight will change the molecules to the excited state, and then when returned to the original energy state their release energy in the form of fluorescence or low heat. This process continues to repeat and is influenced by the number of chromophore groups and electron resonance in these organic compounds.

Polar extracts that are active antioxidants, sunscreens, and contain the highest levels of phenolics were tested invivo using male white mice using a topical application on the dorsal area. The test results can be seen in table 4 and figure 1. On visual observations, increased doses of polar extract further reduced erythema and wrinkles on the skin of test animals. The highest concentration of extract (7.5 %) showed dermis performance similar to the normal control group. The polar extract is applied to the back of the mice with a different dosage group of 2.5, 5, and 7.5%. After two weeks of treatment with UV exposure and the administration of the extract, creams were observed on the skin of the mice visually and histopathologically. On histopathological observations, the UV control group saw high collagen scores and fiboblast scores and epidermis thickening. While in the dose group, there was a decrease in collagen score, fibroblast and epidermal thickness, and the increase in dose and at the highest dose (7.5%). The appearance of the animal dermis approached the normal group that was not exposed to UV light. Epithelial cell scores in the UV control animal group were lower than in other groups.

Previous research in the plant *E. mollis* reveals the activity of sesquiterpene lactone as an anticancer. There has been no study showing the potential of *E. mollis* in overcoming skin disorders caused by UV exposure based on antioxidant bioactivity and its chemical components. The search for antioxidant and photoprotective active agents is urgently needed at this time, where the negative impact of solar radiation is increasing due to global warming.

Based on histopathological observations, exposure to UV light leads to increased collagen deposition, collagen compaction, and thickening of the epidermis (acanthosis). Thickening of the epidermis can be caused due to hyperkeratosis (thickening of the stratum corneum), spongiosis (edema containing intercellular fluid). Thickening of the epidermis due to exposure to UV light can reach 12 times compared to normal skin epidermis.[27] In general, UV light can result in the degradation of collagen fibers. But at the same time, there is also an increase in collagen synthesis by fibroblasts as feedback to physiological disorders. This imbalance between degradation and synthesis processes results in collagen deposition and irregular thickening of fibrils, leading to fibrosis.[28,29] In the group of animals given 7.5% topical *E. mollis* extract, collagen scores and fibroblast scores were the same as the normal group that was not exposed to UV light. That scores indicate the protective effect of polar extract *E. mollis* on the skin so that UV rays do not penetrate further in epidermis and dermis and prevent damage.

Analysis of the chemical components of the polar extract *E. mollis* was conducted by the LC-HRMS method and identified various bioactive chemical components of the phenolic group, flavonoids, and little amino acids, as shown in table 5. Analysis of the chemical components of the polar extract E. mollis was conducted by the LC-HRMS method and identified various bioactive chemical components of the phenolic group, flavonoids, and slight amino acids, as shown in table 5.

The chemical compounds of polar extract *E. mollis* have not been previously reported, so the findings from this study may add to references to the bioactive phytochemical content

of the *E. mollis* plant. Phenolic compounds such as caffeic acid, 3,5-di-O-caffeoylquinic, chlorogenic acid, and flavonoid components such as quercetin, rutin, trifolin, kaempferol, and apigenin have been reported to have antioxidant activity.[25,30]

CONCLUSIONS

This study showed that polar extract *E. mollis* has a high phenolic content and is active as an antioxidant, and organic sunscreen can protect the skin of mice exposed to UV rays. Administration of polar extract *E. mollis* can reduce hyperplasia in the epidermis and decrease disorganized or pathological collagen. Polar extract *E. mollis* rich in polyphenol components can be a natural photoprotector that provides skin protection against UV rays through its activity as an antioxidant and organic sunscreen.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank Perintis Padang Foundation for its support in funding this research

REFERENCES

1. de Jager TL, Cockrell AE, Du Plessis SS. Ultraviolet light induced generation of reactive oxygen species. Adv Exp Med Biol. 2017;996:15–23.
2. Brand RM, Wipf P, Durham A, Epperly MW, Greenberger JS, Falo LD. Targeting mitochondrial oxidative stress to mitigate UV-induced skin damage. Front Pharmacol. 2018;9(AUG):1–10.
3. Young AR, Claveau J, Rossi AB. Ultraviolet radiation and the skin : Photobiology and sunscreen photoprotection. J Am Acad Dermatol. 2017;76(3S1):100–9.
4. Saewan N, Jimtaisong A. Photoprotection of natural flavonoids. J Appl Pharm Sci. 2013;3(9):129–41.
5. Kostyuk V, Potapovich A, Albuhaydar AR, Mayer W, De Luca C, Korkina L. Natural Substances for Prevention of Skin Photoaging: Screening Systems in the Development of Sunscreen and Rejuvenation Cosmetics. Rejuvenation Res. 2018;21(2):91–101.
6. Poon F, Kang S, Chien AL. Mechanisms and treatments of photoaging. Photodermatol Photoimmunol Photomed. 2015;31(2):65–74.
7. Geoffrey K, Mwangi AN, Maru SM. Sunscreen products: Rationale for use, formulation development and regulatory considerations. Saudi Pharm J [Internet]. 2019;27(7):1009–18. Available from: https://doi.org/10.1016/j.jsps.2019.08.003
8. Ruszkiewicz JA, Pinkas A, Ferrer B, Peres T V., Tsatsakis A, Aschner M. Neurotoxic effect of active ingredients in sunscreen products, a contemporary review. Toxicol Reports [Internet]. 2017;4(March):245–59. Available from:

http://dx.doi.org/10.1016/j.toxrep.2017.05.006

9. Downs CA, Dinardo JC, Stien D, Rodrigues AMS, Lebaron P. Benzophenone Accumulates over Time from the Degradation of Octocrylene in Commercial Sunscreen Products. Chem Res Toxicol. 2021;34(4):1046–54.
10. Sardar SW, Choi Y, Park N, Jeon J. Occurrence and concentration of chemical additives in consumer products in Korea. Int J Environ Res Public Health. 2019;16(24):1–15.
11. Nunes AR, Vieira ÍGP, Queiroz DB, Leal ALAB, Maia Morais S, Muniz DF, et al. Use of Flavonoids and Cinnamates, the Main Photoprotectors with Natural Origin. Adv Pharmacol Sci. 2018;
12. Cavinato M, Waltenberger B, Baraldo G, Grade CVC, Stuppner H, Jansen-Dürr P. Plant extracts and natural compounds used against UVB-induced photoaging. Vol. 18, Biogerontology. Springer Netherlands; 2017. p. 499–516.
13. Indradi RB, Fidrianny I, Wirasutisna KR. DPPH Scavenging Activities and Phytochemical Content of Four Asteraceae Plants. Int J Pharmacogn Phytochem Res. 2017;9(6):755–9.
14. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93.
15. Bich Ngoc TT, Hoai Nga NT, My Trinh NT, Thuoc TL, Phuong Thao DT. Elephantopus mollis Kunth extracts induce antiproliferation and apoptosis in human lung cancer and myeloid leukemia cells. J Ethnopharmacol [Internet]. 2020;263(article 113222):1–3. Available from: https://doi.org/10.1016/j.jep.2020.113222
16. Wu Z-N, Zhang Y-B, Chen N-H, Li M-J, Li M-M, Tang W, et al. Sesquiterpene lactones from Elephantopus mollis and their anti-inflammatory activities. Phytochemistry [Internet]. 2017;137:81–6. Available from: http://www.sciencedirect.com/science/article/pii/S0031942217300389
17. Ministry of Health Republic of Indonesia. Indonesian Herbal Pharmacopeia. 2017. p. 526–8.
18. Hatami T, Emami SA, Miraghaee SS, Mojarrab M. Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of artemisia biennis willd. Iran J Pharm Res. 2014;13(2):551–8.
19. Wróblewska KB, Baby AR, Grombone Guaratini MT, Moreno PRH. In vitro antioxidant and photoprotective activity of five native Brazilian bamboo species. Ind

Crops Prod [Internet]. 2019;130(November 2018):208–15. Available from: https://doi.org/10.1016/j.indcrop.2018.12.081

20. Madan K, Nanda S. In-vitro evaluation of antioxidant, anti-elastase, anti-collagenase, anti-hyaluronidase activities of safranal and determination of its sun protection factor in skin photoaging. Bioorg Chem [Internet]. 2018;77:159–67. Available from: http://www.sciencedirect.com/science/article/pii/S0045206817307307
21. Serafini MR, Detoni CB, Menezes PDP, Pereira Filho RN, Fortes VS, Vieira MJF, et al. UVA-UVB Photoprotective Activity of Topical Formulations Containing Morinda citrifolia Extract. Biomed Res Int. 2014;2014.
22. Fatmawaty, Anggreni NGM, Fadhil N, Prasasty VD. Potential in Vitro and in Vivo Antioxidant Activities from Piper crocatum and Persea americana Leaf Extracts. Biomed Pharmacol J. 2019;12(2):661–7.
23. Carmona-Hernandez JC, Taborda-Ocampo G, González-Correa CH. Folin-Ciocalteu Reaction Alternatives for Higher Polyphenol Quantitation in Colombian Passion Fruits. Int J Food Sci. 2021;2021.
24. Al-Mamary MA, Moussa Z. Antioxidant Activity : Presence and Impact of Hydroxyl Groups in Small Molecules of Natural and Synthetic Origin. IntechOpen. 2021;Chapter 13(Antioxidants-Benefits, Sources, Mechanisms of Action):1–28.
25. Korkina L, Kostyuk V, Potapovich A, Mayer W, Talib N, De Luca C. Secondary plant metabolites for sun protective cosmetics: From pre-selection to product formulation. Cosmetics. 2018;5(32):1–20.
26. Hano C. Antioxidant and Anti-aging Action of Plant Polyphenols. MDPI Special Issue. 2020. 1–182 p.
27. Hassan SMA, Hussein AJ, Saeed AK. Role of Green Tea in Reducing Epidermal Thickness upon Ultraviolet Light-B Injury in BALB/c Mice. Adv Biol. 2015;2015:1–6.
28. McKleroy W, Lee TH, Atabai K. Always cleave up your mess: Targeting collagen degradation to treat tissue fibrosis. Am J Physiol - Lung Cell Mol Physiol. 2013;304(11).
29. Mohiuddin AK. Skin Aging & Modern Age Anti-aging Strategies. Glob J Med Res. 2019;19(2):15–60.
30. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules. 2019;24(22):14–6.

Tabel 1. Characterization results of *Elephantopus mollis* extracts

Extract	Characteristics				
	Organoleptic	yield	loss on drying	ash content	Phytochemical compound
Nonpolar extract	Thick, dark green, distinctive odor, slightly bitter taste	2.95 % (28.92 g)	6.73 %	2.31%	Steroids
Semipolar extract	Thick, blackish green, distinctive odorless, slightly bitter taste	3.83 % (37.54 g)	5.68%	4.09%	Terpenoids, phenolics
Polar extract	Thick, yellowish-brown, odorless, slightly bitter taste	4.33 % (42.47 g)	9.40%	6.20%	Alkaloids, phenolic, flavonoids, saponin

Table 2. Test results of antioxidant activity and total phenolic content of *E. mollis* extract

No	Extracts	The IC_{50} value	Total Phenolic Compounds	Pearson's Correlation
1	Nonpolar	363.858 ± 3.046 µg/mL[a]	24.182 ± 0.721 mg/g GAE[a]	-0.864**
2	Semipolar	177.320 ± 1.405 µg/mL[b]	33.187 ± 0.937 mg/g GAE[b]	
3	Polar	46.553 ± 0.149 µg/mL[c]	106.936 ± 0.891 mg/g GAE[c]	

** : significant at p<0.01

Table 3. SPF value of *E. mollis* extract

Wavelength	EE x I	Absorption of extract at a concentration of 100 µg/ml			Absorption of extract at a concentration of 250 µg/ml		
		Nonpolar	Semipolar	Polar	Nonpolar	Semipolar	Polar
290	0.0150	0.196	0.351	0.511	0.471	0.872	1.288
295	0.0817	0.185	0.341	0.534	0.446	0.851	1.352
300	0.2874	0.161	0.341	0.544	0.434	0.844	1.376
305	0.3278	0.173	0.339	0.545	0.424	0.847	1.377
310	0.1864	0.172	0.343	0.548	0.425	0.863	1.390
315	0.0839	0.179	0.348	0.569	0.436	0.868	1.448
320	0.0180	0.184	0.357	0.601	0.451	0.886	1.543
SPF Value		1,72 ± 0.01	3.4 ± 0.01	5.47 ± 0.01	4.31 ± 0.16	8.53 ± 0.03	13.85 ± 0.01

Table 4. Results of examination of skin performance and dermis of test animals

Groups	Collagen score	Fibroblast score	Epitel score	Epidermal thickness	Wrinkle score	Erythema score
Normal control	1.3 ±0.09[a]	1.33 ± 0.47[a]	3 ± 0.00[a]	23.3 ±0.65[a]	0±0.00[a]	0±0.00[a]
UV control	2.8 ±0.16[b]	3.00 ±0.00 [b]	2.3 ±0.47[b]	69.3±1.82[b]	2.28 ± 0.71[b]	3.08 ± 1.09[b]
2.5 % of extract	2.3 ±0.25[c]	2.67 ± 0.47[b,c]	3 ± 0.00[a]	35.4±1.86[c]	1.13 ± 0.48[c]	1.44 ± 0.77[c]
5 % of extract	1.9 ±0.25[c,d]	2.00±0.47[a,b]	3 ± 0.00[a]	29.2±2.13[d]	0.71 ± 0.39[d]	0.81 ± 0.49[d]
7.5 % of extract	1.5 ±0.19[a,d]	1.67 ±0.47[a]	3 ± 0.00[a]	25.1±1.80[a]	0.41 ± 0.23[d]	0.43 ± 0.25[a,d]

Table 5. Chemical component profile of polar extract *E. mollis* with LC-HRMS

No	Name	Formula	Molecular Weight	RT [min]
1	Chlorogenic acid	C16 H18 O9	376.07748	0.865
2	Adenine	C5 H5 N5	135.0547	1.165
3	Isoleucine	C6 H13 N O2	131.09473	1.51
4	Kynurenic acid	C10 H7 N O3	189.04282	4.148
5	(1r,3R,4s,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1-carboxylic acid	C16 H18 O9	354.09539	4.878
6	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	C12 H12 N2 O2	216.09001	5.274
7	(1S,3R,4R,5R)-1,3,4-trihydroxy-5-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}cyclohexane-1-carboxylic acid	C17 H20 O9	368.11098	6.287
8	2-[(4-hydroxy-3,5-dimethoxyphenyl)methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol	C15 H22 O9	368.11098	6.695
9	Rutin	C27 H30 O16	610.15416	7.007
10	Quercetin	C15 H10 O7	302.04265	7.309
11	Quercetin-3β-D-glucoside	C21 H20 O12	464.09596	7.312
12	3-{[(2S,3R,4S,5R,6R)-3,5-dihydroxy-6-(hydroxymethyl)-4-{[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	C27 H30 O15	594.15911	7.596
13	Kaempferol	C15 H10 O6	286.04775	7.606
14	3,5-di-O-caffeoyl quinic acid	C25 H24 O12	516.12699	7.852
15	Trifolin	C21 H20 O11	448.10094	7.894
16	Apigenin 7-O-glucuronide	C21 H18 O11	446.08553	8.151
17	Apigenin	C15 H10 O5	270.05217	8.153
18	Caffeic acid	C9 H8 O4	180.04235	8.199

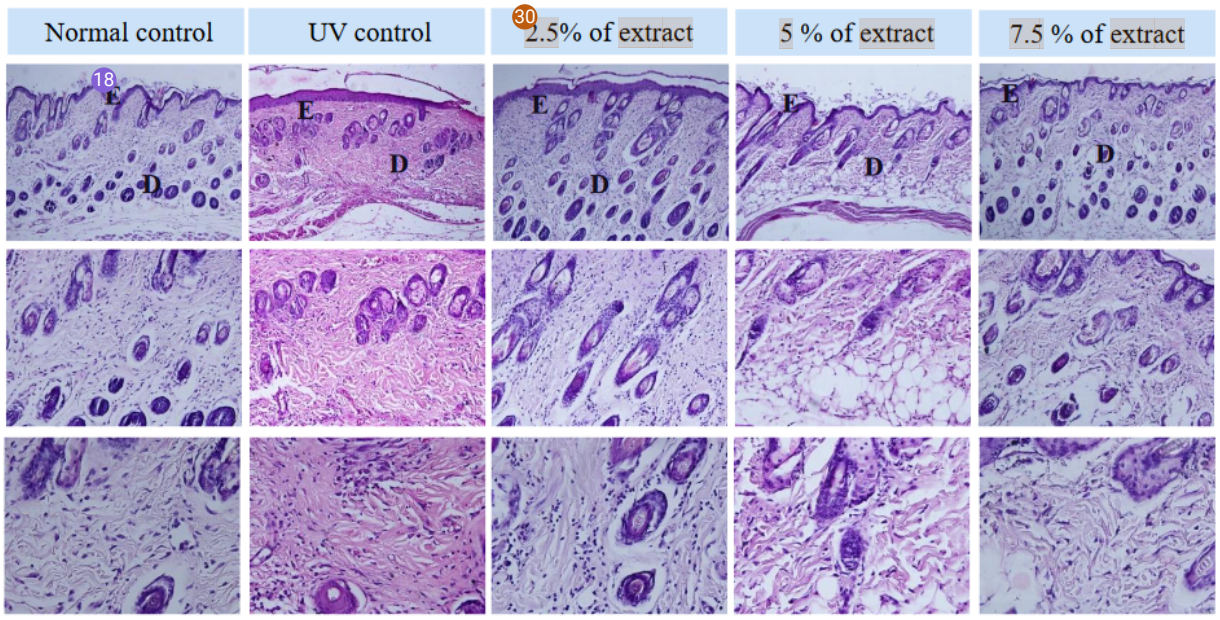

Figure 1. Histology of animal skin tissue shows the epithelium of the epidermis (E) and dermis (D), staining with Hematoxylin-eosin. Top panel objective 10x, middle 20x under objective 40x.