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Original Article

Photoprotective Activities of Standardized *Anredera scandens* (L.) Moq. Leave Extract: An In-Vitro Study on UVB Absorption and UVA Protection

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Abstract

Background: Skin cancer is a worldwide disease caused by exposure to ultraviolet radiation, both UVA and UVB rays. Interest in discovering new natural ingredients with photoprotective activity has increased research to find plants containing high amounts of phenolic and flavonoid compounds.

Objective: This research aimed to assess the in vitro photoprotective activity of ASL (ethanol extract of *Anredera scandens* (L.) Moq. leaves) standardized based on the Indonesian Herbal Pharmacopoeia.

Methods: Extraction was performed using the maceration method with 70% ethanol as the solvent. Parameters used to determine quality are drying loss, water content, total ash content, acid insoluble ash content, total flavonoid content, and total phenolic content. Photoprotective activity against UVB rays was determined by measuring SPF value using the Mansur spectrophotometric method, while activity against UVA rays was tested using the trans-resveratrol degradation model.

Results: Results indicate that ASL extract fulfilled quality requirements based on total flavonoid content (9.983 ± 0.090%) and total phenolic content (13.098 ± 0.611%). The SPF value was increased proportional to the increase in concentration. The Sample showed an SPF value of 5.96 when tested at a concentration of 200 µg/mL. SPF value reached 27.9 at 1,000 µg/mL. The UVA photoprotection assay indicated that the ASL extract provided limited protection against trans-resveratrol degradation, with a reduction of 18.90%, which was lower than the positive control but still significantly better than the negative control.

Conclusion: ASL extract can be developed as a candidate for a natural-based photoprotective active ingredient, used as a single agent or supportive component for sunscreen products.

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INTRODUCTION

Skin cancer continues to be a worldwide epidemic due to its steadily increasing occurrence. The American Cancer Society estimated that there were over 1.6 million new skin cancers diagnosed in the year 2012 alone. These cancers led to 12,190 deaths. Non melanoma skin cancer (NMSC) accounted for about 99% of these cases. The remaining cases, nearly 76,250 new cases, were malignant melanoma and led to over 9,180 deaths.[1] WHO also published global estimates around the same time, which put incidence at approximately 200,000 cases of malignant melanoma and deaths at 65,000 annually. Rising incidence has been attributed to changing patterns of ultraviolet radiation exposure related to contemporary lifestyles, greater recreational time outdoors, and other factors that affect intensity of solar UV exposure.[2–4]

Skin cancer is caused by many factors but the most important of them all is ultraviolet radiation (UVR). According to their wavelength UVRs are divided into three types namely ultraviolet A(UVA), ultraviolet B(UVB) and ultraviolet C(UVC). UVA and minimal UVB rays reach the surface of the earth while UVC is filtered by ozone.[5] Humans are exposed to UVR from both sunlight and artificial sources. Artificial UVR is used for industrial and cosmetic purposes and can contribute to a person's overall skin exposure.[6] Chronic UVR exposure can induce ROS formation. Lipid peroxidation, alteration of cellular structural proteins, mutations in DNA, inflammation and apoptosis are all effects of ROS which contribute to photocarcinogenesis.[7,8]

One way to protect skin from damaging effects of UVR is with topical applications of photoprotective molecules or sunscreens. Photoprotective products work depending on the photoprotective agents' ability to filter UV radiation

and prevent its penetration into the skin.[9,10] The majority of sunscreen products available on the market today are formulated with chemical UV filters. Although safe and effective, some chemical filters have shown safety concerns such as endocrine disruption, phototoxicity and possible carcinogenicity when studied under certain conditions.[11] Benzophenone, which is commonly used in sunscreen products, has caused cancer in several organs of experimental animals when exposed to chronically. [12]

Driven by concerns over safety and sustainable production, current trends in photoprotection science have explored natural products as alternative sources of UV-filters. Flavonoids and polyphenols are plant secondary metabolites with conjugated aromatic chemical structures, which allow these substances to readily absorb UV radiation. [13,14] In addition to acting as natural chromophores, these antioxidants also demonstrate effective ROS inhibition and can protect skin cells against oxidative stress caused by UVA and UVB radiation exposure. [15–17] Furthermore, it was recently reviewed that polyphenols derived from plants can confer broad-spectrum UV protection and reduce inflammatory reactions and skin cell apoptosis within the epidermis. [18–20] Recent studies have further emphasized that plant-derived polyphenols act as multifunctional photoprotective agents by combining UV absorption capacity with antioxidant-mediated cellular protection. [18–20]

Binahong or *Anredera scandens* (L.) Moq. leaves (ASL) are leaves of tropical medicinal plants which have been used for many years in folk medicine. It has been reported that it contains many biologically active compounds including flavonoids and phenolic compounds that have potent antioxidant activity. Earlier studies managed to isolate flavonol compounds from ASL that displayed potent free radical scavenging activities with IC_{50} values falling under strong antioxidant activities.[21,22] As for toxicity profiling, acute skin irritation tests conducted using 70% ethanol extract of ASL showed no lethality even at 5,000 mg/kg BW implying its safe topical application. [23,24]

Relationship between antioxidant capacity and photoprotective activity has already been established. Radical scavenging capacity of phenolic-rich plant extracts often shows correlation with in vitro photoprotective potential indicated by SPF values and biological photoprotection against UVA radiation.[25–27] Phenolics have also been shown to indirectly protect against UVA by quenching oxidative stress and free radical generation. [28] However, despite the well-documented antioxidant properties of *Anredera scandens*, there is still limited information regarding its specific photoprotective activity, particularly in relation to both UVB absorption and UVA protection mechanisms. However, to date, there has been no study specifically evaluating the in vitro photoprotective activity of *Anredera scandens* (L.) Moq. leaf extract in terms of UVB and UVA protection parameters.

Therefore, based on the above-mentioned studies, we can propose that the photoprotective effect of ASL extract can be attributed to its antioxidant activity. Photoprotective activity evaluation in vitro is justified as a first approach to verify this hypothesis. This assay includes the determination of SPF value as a measure of UVB protection ability and testing of UVA inhibition using trans-resveratrol degradation assay. The spectrophotometric method proposed by Mansur (1986) will be used because it's easy, reproducible and commonly used for evaluation of photoprotective activity of plant-derived sunscreen candidates.[14,29,30] Therefore, the aim of this study was to evaluate the in vitro photoprotective activity of *Anredera scandens* (L.) Moq. leaf extract by determining its SPF value and UVA inhibition capacity.

METHODS

Study Design

Experimental design: Laboratory research was conducted to determine the photoprotective activity of a standardized extract from ASL (*Anredera scandens* (L.) Moq. leaves). Stages of the research included plant material preparation, extract standardization, total flavonoids and total phenolic content determination, assessment of UVB photoprotective activity by determining SPF value, assessment of UVA photoprotective activity using trans-resveratrol degradation model and statistical analysis of the obtained results.

Research Instruments

Analytical balance (AND®, Japan), rotary evaporator (Eyela®, Japan), drying oven, centrifuge, UV–Visible spectrophotometer (Genesys®, Thermo Scientific), porcelain crucibles, mortar and pestle, porcelain dishes, sedimentation tubes, volumetric flasks, volumetric pipettes, micropipettes and common laboratory glassware (Iwaki Pyrex®) were used in this work. Ultraviolet A irradiation was produced by UV lamp (wavelength range of 320–400 nm).

Materials

Raw material used was ASL powder, which was obtained from Hargobinangun, Pakem, Sleman Regency, Special Region of Yogyakarta. CV. Merapi Farma Herbal were suppliers of the raw material. Authentication of plant specimen was done previously, so the reidentification wasn't conducted. Solvents used for extraction were ethanol 70% and 96% (Bratachem®). Standardization and analysis reagents were aluminum chloride (Merck®), sodium acetate (Merck®), hydrochloric acid 37% (Merck®), rutin standard (Merck®), gallic acid (Sigma®), Folin–Ciocalteu reagent (Sigma®), sodium hydroxide (Sigma®), trans-resveratrol (Sigma®), and distilled water. All chemicals were used as analytical grade unless they were stated differently. Positive control used for UVA photoprotection assay was a sunscreen preparation bought commercially (Soltan Boots®).

Determination of Loss on Drying of ASL Powder

Loss on drying was performed gravimetrically according to Indonesian Herbal Pharmacopoeia method. Approximately 2 g of powdered sample was accurately weighed into a dried and tared weighing bottle. Drying was performed by placing the bottle (sample inside) in oven at 105°C for 30 minutes. The bottle was cooled in desiccator and weighed before sample was introduced into bottle. Sample was spread evenly until it formed a thin layer inside bottle, then dried in oven at 105°C with bottle caps loosened for 30 minutes. The bottle was capped tightly, cooled in desiccator for 30 minutes, and weighed. The drying and weighing cycle continued until constant weight was achieved (difference between two successive weighings ≤ 0.25%). Loss on drying (%) was determined as percentage decrease in sample weight divided by original mass of powdered material[31].

Preparation of ASL Extract

A total of 100 g of ASL powder was macerated with 70% ethanol at a ratio of 1:10 (w/v) for 24 hours with occasional stirring. Separated by filtration, residue was remacerated twice with same method. All filtrates were combined and then concentrated with rotary evaporator at 68°C until it became viscous extract. Extract yield percentage was determined and compared with minimum Indonesian Herbal Pharmacopoeia requirement which state yield not less than 11.91%.[31]

Extract Standardization

Extract standardization involved analyses for moisture content, total ash, acid-insoluble ash and total flavonoid content as described in the Indonesian Herbal Pharmacopoeia.[31] Moisture content was evaluated gravimetrically at 105°C until constant weight was reached.[32] Total ash and acid-insoluble ash were measured by gradual incineration followed by treatment with dilute hydrochloric acid. Total flavonoid content was quantified spectrophotometrically using aluminum chloride complexation method. Rutin was used as reference standard and absorbance was measured at 425 nm.

Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, an aliquot of extract solution was mixed with aluminum chloride solution ($AlCl_3$) and sodium acetate. The mixture was incubated at room temperature for a specified time to allow complex formation. Absorbance was measured at 425 nm using a UV–Visible spectrophotometer. Rutin was used as a reference standard, and total flavonoid content was expressed as percentage of rutin equivalent (% w/w). Calibration curve was constructed using rutin standard solutions, and flavonoid content was calculated based on the regression equation obtained.[31]

Determination of Total Phenolic Content

The Folin–Ciocalteu method was used to determine the total phenolic content of the standardized extract, using gallic acid as the standard. Readings were performed at a wavelength maximum of 730 nm. Results were expressed as milligrams of gallic acid equivalents per gram of extract.[31]

Evaluation of UVB Photoprotective Activity

UVB photoprotective activity was evaluated by determining SPF. SPF value was calculated according to Mansur method. Extract solutions were diluted in 70% ethanol at concentration ranging from 200 to 1,000 µg/mL. The absorption was read from 290 to 320 nm at interval of 5 nm. SPF values were calculated using Mansur equation and multiplying by a correction factor of 10.[26,29,33]

Evaluation of UVA Photoprotective Activity

UVA photoprotective activity was measured using a trans-resveratrol photoprotection assay. In the photoprotective assay, ASL extract was tested at a concentration of 1 mg/mL, in combination with 10 µg/mL trans-resveratrol solution. Conditions were optimized for UVA induced degradation of trans-resveratrol by irradiating a 10

µg/mL solution of trans-resveratrol with UVA light (320–400 nm) for 15 min to 120 min. For the photoprotective assay, trans-resveratrol degradation with test extract protection, sunscreen protection, negative controls and dark control were measured. Percent degradation was determined by UV spectrophotometry at 306 nm.[9,25,28]

Statistical Analysis

All experiments were performed three times, and results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate statistical software. Prior to comparative analysis, data distribution was assessed using the Shapiro–Wilk normality test. For SPF evaluation, linear regression analysis was applied to determine the correlation between extract concentration and SPF values. For UVA photoprotective activity, differences between groups (normal control, negative control, positive control, and ASL extract group) were analyzed using one-way analysis of variance (ANOVA), followed by a post hoc Least Significant Difference (LSD) test to identify statistically significant differences between groups. A p-value of less than 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Sample Preparation

ASL samples were collected from Hargobinangun, Pakem District, Sleman Regency, Special Region of Yogyakarta. The sampling was assisted by CV. Merapi Farma Herbal. The samples were sorted and redried to eliminate other parts of plants and remaining contaminants that stuck on the ASL simplicia. Samples were sorted and redried to reduce moisture content and homogeneity of the batch before extraction. Moisture content and raw material quality were controlled to maintain stability of phenolic compounds that could degrade during storage and extraction process.[34–36] The dried material was pulverized using a mill to produce a fine ASL powder. Increasing surface area allows for greater exposure of solvent. By creating more area for solvents to come in contact with the plant material, secondary metabolites have an easier time diffusing into the extraction solvent. Improving extraction efficiency will have an effect on the recovery of phenolic and flavonoid compounds, leading to an increase in photoprotective activity of the ASL extract.[37,38]

Determination of Loss on Drying of ASL Powder

Loss on drying of ASL powder was determined by a gravimetric method. Materials containing essential oils should not be subjected to drying processes where active constituents may volatilize due to excessive heat application. Literature sources suggest that ASL is free of essential oils and therefore gravimetric determination should not entail excessive losses of bioactive compounds[39,40]. Loss on drying was found to be 8.048 ± 0.109% w/w, which is lower than the pharmacopeial maximum limit of 10% by the Indonesian Herbal Pharmacopeia. Since the loss on drying value was controlled, it is suggested that drying of the sample did not subject nonvolatile contents to excessive loss. Drying is an important consideration since degradation of phenolic compounds that elicit photoprotective activity can occur when submitted to prolonged heating or overheating.[17,41,42]

Preparation of ASL Extract

ASL powder was extracted by maceration using 70% ethanol. This concentration was chosen because ethanol in that concentration is able to solubilize polar to semipolar compounds such as flavonoids, tannins, and other phenolic compounds which have been shown to be important players in photoprotection.[8,16,43,44] The extraction yield was found to be 22.103% w/w or 110.518 g of extract/500 g ASL powder. The percentage yield obtained is higher than minimum requirement yields of not less than 11.91% indicated by Indonesian Herbal Pharmacopoeia. High yield generally indicates that the solvent system used was capable of extracting a good percentage of chemical constituents present in the plant material. High extract yields are usually correlated with high number of bioactive compounds present which may contribute to good photoprotective performance of the ASL extract.[25,45]

Determination of Moisture Content of ASL Extract

Moisture content determination was also included as extract quality determination parameters. High moisture levels will impact negatively on extract stability and also increase the risk of microbial growth that may reduce the extract's biological activity. Increased water activity may also impair dose dependability and reproducibility of biological effects. Water content in the 70% ethanolic extract of ASL was determined as 3.8265 ± 0.0978% w/w, which was below maximum allowance of 8.85% w/w set by Indonesian Herbal Pharmacopoeia. Low moisture content helps ensure stability of phenolic and flavonoid compounds responsible for ultraviolet absorption resulting in reliable photoprotective efficacy.[5,25]

Determination of Total Ash Content of ASL Extract

Total ash determines the amount of residue obtained from incineration; It is useful to know the quantity of inorganic substance. Total ash can indicate the nature and degree of mineral content found in the plant or level of contamination by mineral matters from the surroundings. Total ash value of ASL extract is 0.239 ± 0.048% w/w. Total ash fulfilled Indonesian Herbal Pharmacopoeia standards which limited to 1.64%. Low amount of total ash indicated low mineral contamination. High contamination may disrupt UV absorbance value which will affect photoprotective property assessment.[4,34]

Determination of Acid-Insoluble Ash Content of ASL Extract

The acid-insoluble ash content was analyzed to determine the amount of insoluble residues, which are likely to represent silica contamination. The silica impurities may be derived from sand or soil which contaminates ASL during harvest. Increased silica content may contribute to skin irritation and may interfere with photoprotection parameters specifically erythema formation.[4,34] Acid insoluble ash content was found to be 0.0261 ± 0.0021% w/w which was below the acceptable limit of 0.05% according to Indonesian Herbal Pharmacopoeia. This demonstrated that there was minimal to no interference of other inorganic materials during preparation and extraction.

Determination of Total Flavonoid Content of ASL Extract

The total flavonoid content was measured as quality control parameter as well as measurement of extract's contribution to photoprotection. Flavonoids are phenolic compounds that contain conjugated aromatic group that able to absorb UV radiation as well as act as antioxidants. ASL extract had total flavonoid content of 9.983 ± 0.090% w/w, calculated as rutin equivalent. The minimum value that satisfied by this extract was 8.96% according to Indonesian Herbal Pharmacopoeia. High enough flavonoid content justifies ASL extract's capacity in absorbing UVB and partially UVA rays. It had been proven that flavonoid compounds like quercetin and rutin could greatly contribute to SPF value elevation as well as suppress oxidative stress caused by UV radiation.[8,16,30]

Determination of Total Phenolic Content of ASL Extract

Total phenolic content was measured since phenolic compounds are thought to be primarily responsible for photoprotective activity. Total phenolic content was measured using the Folin–Ciocalteu method according to the Indonesian Herbal Pharmacopoeia. The ASL extract was determined to have a total phenolic content of 13.098 ± 0.611% w/w, or 130.98 µg/mg extract when calculated as gallic acid equivalents. This value was similar to that which was obtained for flavonoid content. The total phenolic content was greater than the flavonoid content indicating that other phenolic compounds such as tannins may be present in the extract. Phenolic compounds have been shown to absorb ultraviolet light as well as scavenge free radicals produced when exposed to UV radiation. Total phenolic content has been positively correlated with in vitro SPF values as well as protective activity against UVB radiation in other plant extracts.[25,26,33,40,43] The relatively high phenolic content observed may account for photoprotective efficacy of the ASL extract at lower concentrations.

Determination of Sun Protection Factor (SPF) of ASL Extract

SPF values for the standardized ASL extract increased in a dose-dependent manner (**Table 1**). This suggests that there is a direct correlation between concentration of active ingredients and ability of the extract to absorb UVB rays. Absorbance being dependent on concentration follows basic principles of in vitro photoprotection.

Table 1. Results of Determining the SPF value of ASL Standardized Extract

Concentration (µg/mL)	SPF
200	5.96
400	11.58
600	16.46
800	22.7
1,000	27.9

The SPF values achieved suggest that ethanolic ASL extract comply with requirements for UVB photoprotection activity. Minimum UVB protection can be conferred by SPF ≥ 6 while SPF ≥ 15 can be considered moderate to good UVB photoprotection as per ASEAN Sunscreen Labelling Guidelines and FDA rules. Regression analysis revealed that standardized ASL extract could reach SPF 6 at lower concentrations while it has the potential to achieve SPF 15 within concentrations feasible for cosmetic preparations. The photoprotective effect can be attributed to the high content of phenolic and flavonoids in the extract. Phenolics contain conjugated double-bond systems which serve as chromophores and are known to be efficient UVB absorbers. Positive correlation between total phenolic content and observed in vitro

SPF values has been noted in various plant extracts.[25,26,30] When compared to earlier researches UVB photoprotection offered by ASL extract analyzed in this study seems better. Ethanolic extract of Spondias purpurea fruit yielded SPF 15 at the concentration of 5 mg/mL.[25] Standardized ASL extract has shown the capability of yielding similar SPF at lesser concentrations. Lesser concentration required to achieve particular SPF value indicates greater photoprotective potency. Results clearly indicate that ASL extract can be a potential active ingredient for herbal sunscreen formulations.

Evaluation of Photoprotective Activity Against UVA Radiation

Photoprotection assay results against UVA radiation listed in **Table 2** revealed that standardized ASL extract afforded partial photoprotection of resveratrol. Loss of resveratrol absorbance by the test sample group (18.9%) was significantly less than negative control A and B where absorbance fell over 60%. These results indicate that neither 70% ethanol afforded photoprotection nor contributed to photoprotection observed in the test sample group as seen to be caused by the bioactive compounds in the ASL extract. The observed UVA protection may be attributed to the antioxidant mechanism of phenolic and flavonoid compounds, which are capable of scavenging reactive oxygen species generated during UVA exposure, thereby reducing oxidative degradation processes.

Table 2. Absorbance Result After UVA Photoprotection Activity Test Radiation

Group	Mean Absorbance (± SD)	Mean Decrease (± SD)	Percentage Decrease (%)
Normal Control	0.683 ± 0.004	0.005 ± 0.004	0.73
ASL Extract (1mg/mL)	0.558 ± 0.007	0.130 ± 0.007	18.90
Negative A	0.263 ± 0.003	0.424 ± 0.003	61.68
Negative B	0.271 ± 0.003	0.416 ± 0.003	60.52
Positive Control	0.650 ± 0.002	0.037 ± 0.002	5.48

Note:
1. Normal Control: Resveratrol was unprotected and not irradiated.
2. ASL Extract (1 mg/mL): Resveratrol was protected with the test extract and irradiated.
3. Negative A: Resveratrol was unprotected and irradiated.
4. Negative B: Resveratrol was protected with 70% ethanol and irradiated.
5. Positive Control: Resveratrol was protected with a commercially available product and irradiated.

Resveratrol was chosen as the marker compound due to its high sensitivity to UVA and high photo-oxidative instability. Less reduction in absorbance in the ASL-treated extract implies that degradation was slowed by the extract. This can be attributed to the antioxidative capabilities of phenolic and flavonoid antioxidants that can neutralize reactive oxygen species produced during UVA irradiation. ASL extract was found to have lower UVA photoprotection than the commercial comparator; however, this was significantly different using the LSD statistical test ($p < 0.05$). Thus, it can be concluded that ASL extract shows potential as a natural UVA photoprotective agent but not to the extent of providing ultra-high protection like many commercial products. This is understandable considering commercial products usually utilize blends of the most effective man-made UV filters in robust formulation platforms. These results correlate with previous results which have shown that phenolic rich plant extracts confer UVA protection indirectly through their ability to quench oxidative stress.[9,28] Therefore, ASL extract could potentially be formulated into an adjuvant or booster ingredient for plant-derived sunscreen products to improve UVA protection.

Limitation

This study has several limitations that should be considered when interpreting the results. The photoprotective activity testing was conducted in vitro, so it does not fully represent the complex biological conditions of human skin. It also did not consider the penetration and stability of the compounds in topical formulations. Furthermore, this study did not identify the specific active compounds that play a dominant role in photoprotection, and in vivo testing was not conducted. Therefore, further research is needed to confirm its effectiveness and safety.

CONCLUSION

The standardized extract of *Anredera scandens* (L.) Moq. leaves demonstrated dose-dependent UVB photoprotective activity, as indicated by increasing SPF values, and exhibited partial protection against UVA-induced degradation. These effects are likely associated with its phenolic and flavonoid content. The findings suggest that the extract has potential as a natural photoprotective agent, particularly as a supportive component in sunscreen formulations. These results indicate a correlation between antioxidant-related compounds and photoprotective activity, particularly in UVB absorption and indirect UVA protection. Overall, the extract shows promise as a complementary natural ingredient in photoprotective formulations, although further studies are required to confirm its efficacy in advanced models.

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GENERATIVE AI DISCLOSURE STATEMENT

The authors confirm that no Generative Artificial Intelligence (AI) or AI-assisted technologies were utilized in the writing, data analysis, or preparation of this manuscript.

AUTHOR CONTRIBUTION STATEMENT

Putu Oka Samirana: Conceptualization, Methodology, Validation, Writing—Original Draft, Supervision, Project administration, Funding acquisition. **I Gusti Agung Anom Krisna Nugraha:** Data Curation, Writing–Review & Editing, Visualization. **Anak Agung Gede Rai Yadnya Putra:** Methodology, Supervision, Writing–Review & Editing, Data Curation, Formal Analysis.

CONFLICT OF INTEREST

The authors state that they have no conflicts of interest and that this study received no financial support.

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