Trop J Nat Prod Res, August 2025; 9(8): 3854 - 3859 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

UV-Vis Spectroscopic and Chemometric Analyses for SPF Prediction: Evaluating the Sun Protection Potential of Acacia Leaves Extracts from Different Species, Locations, and Solvent Extractions

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ARTICLE INFO

Article history:
Received 27 March 2025
Revised 14 June 2025
Accepted 02 August
Published online 01 September 2025

ABSTRACT

In recent years, there has been growing interest in the use of plant-based ingredients as natural sunscreen agents. This study aimed to investigate the Sun Protection potential of Acacia extracts using UV-Vis spectroscopy combined with chemometric analysis. Extracts from two Acacia species (Mangium and Auri), sourced from two different locations (BATOLA and BJB), were prepared using ethanol (96%), ethyl acetate, and n-hexane as extraction solvents. The sun protection potential of the extracts was evaluated by determining the sun protection factor (SPF) using UV-Vis spectrophotometric method, followed by chemomimetric analysis. The results showed that the polarity of the solvent for extraction, the species of plants, and the geographical origin significantly influenced SPF values and the extraction of UV-absorbing bioactive compounds. Ethyl acetate extracts showed the highest SPF values (25.43-33.44), particularly in Mangium BJB (31.84) and Auri BJB (30.57), suggesting a greater photoprotective potential. Principal component analysis (PCA) successfully differentiated extracts, while Partial Least Square (PLS) regression ($R^2 > 0.99$) accurately predicted SPF values, validating the reliability of chemometric models for SPF estimation. This study showed that UV-Vis spectroscopy, combined with chemometric modelling, provided a rapid, accurate and non-invasive method to evaluate plant-based sunscreen ingredients, thereby supporting the development of natural and sustainable photoprotective products. Moreover, future studies should focus on compound identification (LC-MS), *in vivo* SPF validation, and development of sunscreen formulations to improve ecofriendly UV protection strategies.

Keywords: Sun Protection Factor, Acacia extracts, UV-Vis spectroscopy, Principle Component Analysis, Partial Least Square Regression.

Introduction

Exposure to sunlight is essential for vitamin D synthesis and overall health maintenance. However, excessive exposure to ultraviolet (UV) radiation can lead to skin damage, premature aging, and an increased risk of skin cancer.[1] To mitigate these risks, sunscreens play a crucial role in shielding the skin from harmful UV radiation, with their efficacy commonly measured by Sun Protection Factor (SPF). SPF quantifies a sunscreen's ability to protect against UV-B rays, the primary cause of sunburn and skin damage.[2] In recent years, there has been growing interest in the use of plant-based ingredients as natural sunscreen agents. This transition is largely driven by concerns over side effects associated with synthetic sunscreen, including skin irritation, allergic reactions, and environmental toxicity.[3] As a result, natural sunscreen formulations are gaining attention due to their safer, skin-friendly, and eco-friendly properties. Studies have shown a strong correlation between the photoprotective effect of plant extracts and their phenolic content.[4]

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Citation: Triyasmono L, Afna DN, Sari RM, Sajidah DPH, Normaidah. UV-Vis Spectroscopic and Chemometric Analyses for SPF Prediction: Evaluating the Sun Protection Potential of Acacia Leaves Extracts from Different Species, Locations, and Solvent Extractions. Trop J Nat Prod Res. 2025; 9(8): 3854 – 3859 https://doi.org/10.26538/tjnpr/v9i8.46

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Among the native Indonesian plants, Acacia species is known for its high phenolic compound content, serving as a promising candidate for natural UV protection.[5] However, the phenolic composition of plants is influenced by several factors, including geographical location, species, and extraction methods.[6] Understanding these factors is critical for optimizing SPF potential of plant-based sunscreens.

The conventional determination of SPF has relied on *in vivo* and *in vitro* methods, including spectrophotometric analysis and human skin testing.[7] However, the increasing interest in natural sunscreens necessitates the development of rapid, non-invasive, and highly accurate SPF prediction methods. Advanced spectroscopic methods, including UV-Vis,[8] Fourier Transform Infrared (FTIR),[9] and Near-Infrared (NIR) spectroscopy[10] have been extensively applied to predict SPF values. These methods are particularly effective because of their ability to capture the absorption characteristics of bioactive sunscreen compounds. Moreover, the integration of chemometric modelling with spectroscopic data has been shown to enhance the accuracy and efficiency of SPF prediction.

Despite the growing interest in plant-derived sunscreens, there is limited report on how growth location, species differences, and extraction methods influence SPF profile of Acacia leaves. On the other hand, this study uniquely integrates UV-Vis spectroscopy and chemometric modelling to predict SPF potential from Acacia leaf extracts, while systematically evaluating the influence of species, growth location, and extraction methods—an area previously underexplored in natural sunscreen research. Therefore, this study aimed to develop a predictive model for SPF potential by analyzing three different extracts from two Acacia species using UV-Vis spectroscopic data combined with chemometric approaches.

Materials and Methods

Plant collection and identification

Acacia leaves were collected in November-December 2023 from Barito Kuala (BATOLA); (-3.177^0 N, 114.66^0 E) and Banjarbaru (BJB); (-3.474^0 N, 114.80^0 E) Districts of South Kalimantan Province, Indonesian. Leaves of the two species of *Acacia mangium* (Mangium) and *Acacia auriculiformis* (Auri) plants were authenticated at the Biology Herbarium Laboratory, FMIPA, Lambung Mangkurat University. The plant samples were assigned the following voucher numbers; 247/LB.LABDASAR/VIII/2023 and 248/LB.LABDASAR/VIII/2023, for *Acacia mangium* and *Acacia auriculiformis* A.Cunn. ex Benth, respectively. Furthermore, the leaves were cleaned with tap water and air dried at 25-30^o C in a shady place for 30 days.

Preparation of extracts

Powdered acacia leaves (500 g each) were extracted separately by maceration in 1 L each of ethanol (96%), ethyl acetate (≥99.5%), and n-hexane (≥99%) at room temperature (25-30^{o}C) for 3 x 24 hours and stirred every 8 hours. The initial solvent was removed, and the marc was re-macerated with the same solvents every 24 hours to avoid saturation and ensure complete extraction of secondary metabolites.[11]

Determination of SPF in vitro

Each sample extract at a concentration of 800 ppm was used for UV-Vis spectroscopic measurements.[12] After preparation, all samples were scanned at wavelengths in the ultraviolet B (UVB) range (between 290 and 320 nm), at every 5 nm interval, and three replicates were performed at each point. At the end of all measurements, the SPF value of each extract was determined using the Mansur equation (1).[13]

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \ldots\ldots (1)$$

Where CF = 10 (Correction Factor), EE(λ) = Erythemogenic Effect of radiation at wavelength λ, I (λ) = Intensity of solar light at wavelength λ, and abs(λ) = Absorbance of wavelength λ by a solution of the preparation. The summation is over the wavelength range from 290 to 320 nm at a 5 nm interval.

UV-Vis spectroscopic measurement and preprocessing

UV-Vis spectroscopic measurements of the diluted ethyl acetate leaf extracts of the two acacia species originating from two different districts were done using UV-Vis double beam spectrophotometer equipped with a quartz cell with an optical path of 1 cm (Orion Aquamate 8100, Thermo Scientific, Germany). Measurements were done in the wavelength range of 280-400 nm with a resolution of 0.5 nm, and in three replicates (Table 1) pre-processed using standard normal variate (SNV) as scatter correction.[12]

Table 1: Number of replicates of all samples collected

Sample	Replicate (n)		
	Ethanol (96%) Extract	Ethyl Acetate Extract	n-Hexane Extract
Mangium	3	3	3
BATOLA			
Mangium BJB	3	3	3
Auri BATOLA	3	3	3
Auri BJB	3	3	3
Total Sample	12	12	12

Note: BATOLA : Barito Kuala; BJB: Banjarbaru

Data analysis

Data matrix from 280-400 nm, consisting of 488 variables from three replicates, was used to build a discrimination model using Principal Component Analysis (PCA). Furthermore, for prediction of SPF value, Partial Least Square (PLS) was used. The leave-one-out cross-validation procedure was used to verify the calibration and prediction model. Finally, the total calibration, validation, and prediction errors (RMSEC, RMSEV, and RMSEP) for each sample model were evaluated. All multivariate analyses were performed in Minitab 17 version 2017, LLC.USA

Results and Discussion

SPF values of Acacia extracts

Table 2 shows the SPF values of the various plant extracts, namely; Mangium and Auri from two locations; BATOLA and BJB. These extracts were prepared using three different solvents, including ethanol (96%), ethyl acetate, and n-Hexane. SPF values indicate the effectiveness of each extract in absorbing or blocking ultraviolet (UV) radiation, which is a critical parameter in evaluating their potential as natural sunscreen agents.[14] The ethyl acetate extracts had the highest SPF values, notably observed in Mangium BJB (31.84) and Auri BJB (30.57). The SPF values of the n-hexane extracts significantly varied among the different samples, with Auri BJB showing the highest SPF (25.57), and Mangium BATOLA the lowest (11.52). The 96% ethanol extract reliably yielded moderate SPF values, with Mangium BJB (SPF = 19.51) attaining the highest absorption in this category. These results indicate that semipolar solvents like ethyl acetate show superior efficacy in extracting UV-absorbing compounds. In comparison, polar solvent (ethanol) and nonpolar solvent (n-hexane) showed selective extraction efficiencies.

Table 2: Actual SPF values of all samples

Sample	SPF Value		
	Ethanol (96%) Extract	Ethyl Acetate Extract	n-Hexane Extract
Mangium	18.20	25.66	11.53
BATOLA	18.21	25.43	11.52
	18.23	25.94	11.53
Mangium	19.43	31.25	13.41
BJB	19.51	31.15	13.39
	19.44	31.84	13.36
Auri	16.44	26.66	18.94
BATOLA	16.41	26.60	18.93
	16.44	26.51	18.92
Auri BJB	17.43	30.57	25.45
	17.50	30.12	25.46
	17.50	29.78	25.58

Table 3 presents a summary of the various solvents extract distinct classes of bioactive compounds, that could affect their capacity to absorb UV radiation. In addition, the result implies that the type of species and their geographical origin affect SPF values. Overall, Mangium BJB showed the highest SPF value of 31.84 in the ethyl acetate extract. This indicates that Mangium from BJB is rich in potent UV protective substances. Similarly, Auri BJB showed significant SPF values in both ethyl acetate (30.57), and n-hexane extract (25.57), signifying the presence of more lipophilic UV-absorbing compounds. BJB typically showed superior SPF values relative to BATOLA samples across various solvents. This observation implies that environmental factors, such as climate, soil composition, and UV exposure in the cultivation region, play a significant role in influencing

the production of secondary metabolites, thereby affecting SPF value.[16] The results are consistent with previous studies, indicating that geographical variation markedly affects the phytochemical content and photoprotective properties of plants.[17]

Table 3: Representative secondary metabolites that could contribute to SPF of all extracts

Extract	General SPF Trend	Possible bioactive compounds contributing to SPF[15]
Ethanol 96%	16.41 – 19.51	Polyphenols, flavonoids, tannins
Ethyl Acetate	25.43 – 31.84	Flavonoid aglycones, alkaloids, terpenoids
n-Hexane	11.52 – 25.57	Carotenoids, tocopherols, lipophilic antioxidants

UV-Vis spectra data

UV-Vis spectra (Figure 1) show the absorbance characteristics of different Mangium and Auri extracts collected from two geographical locations (BATOLA and BJB). The spectra cover the wavelength range of 250 to 400 nm, which is typically associated with the absorbance of bioactive compounds such as flavonoids, phenolics, and other secondary metabolites absorbing UV rays.[18]

A prominent peak appeared at the wavelength range of 285–300 nm, particularly in Mangium BJB (orange) and Auri BATOLA (light blue) samples. This absorption region is typically associated with polyphenolic compounds, flavones, flavonols, and other UV-absorbing phytochemicals.[19] Moreover, the intensity of the peak suggests higher concentrations of UV-absorbing compounds in these samples.

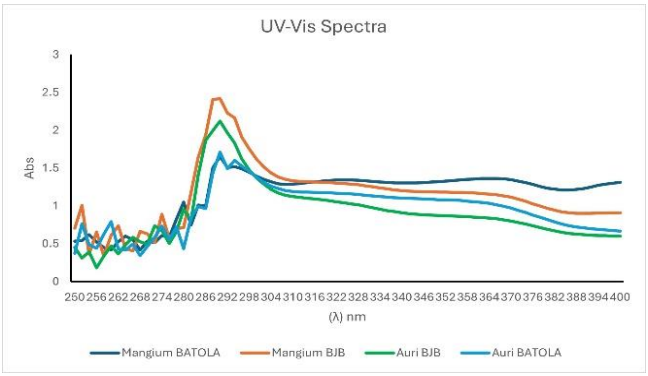

Figure 1: Representative UV-Vis spectra of acacia leaf ethyl acetate extract from two districts

A steady decline in absorbance was observed beyond 300 nm, although certain extracts such as Mangium BATOLA maintained a moderately high level of absorption. This high absorbance suggests the presence of conjugated systems, like quercetin, kaempferol, or other flavonoids, which aid in UV protection.[20] Enhanced absorption in this range is favoured for sun-protection formulations, as it implies the presence of compounds capable of blocking ultraviolet A (UVA) and UVB rays.

Further observation showed variations in absorbance intensities in the same species (e.g., Mangium BATOLA vs. Mangium BJB), suggesting that geographical differences impact metabolite composition. Environmental factors, including soil nutrients, sunlight exposure, and climatic conditions, may contribute to the biosynthesis of these UV-absorbing compounds.[17] The strong UV absorption observed in Mangium BJB and Auri BATOLA indicates a potential high concentration of compounds with high SPF, making these species promising candidates for natural sunscreen formulations. UV-absorbing plant extracts can serve as natural photoprotectants by reducing oxidative stress and preventing UV-induced skin damage.[21]

The impact of geographical location on phytochemical content is significant. In line with the results, higher UV absorption in certain samples implies that geographical and climatic conditions influence the secondary metabolite profile. Plants subjected to elevated UV radiation might produce more UV-protective compounds as a defence mechanism.[16] The differences in bioactive compound content between BATOLA and BJB samples could be examined further using high-performance liquid chromatography (HPLC) or LC-MS analysis to quantify specific bioactive compounds.

Discrimination of the two species from two origins using three solvents with chemometric analysis

UV-Vis spectroscopy combined with chemometrics are increasingly being used to identify and discriminate closely related medicinal plants. Meanwhile, multivariate analysis requires sample preparation that produces reproducible spectroscopic data.[22] This study observed variations in wavelength and intensities in the 32 spectra, and chemometric analysis was used to differentiate the four samples (comprising 2 species from 2 locations, each extracted with 3 different solvents). For constructing the discrimination model, the absorbance measurement spanning 280-400 nm was used as a variable, producing a dataset composed of 32 objects by 61 variables.

Spectroscopic data is customarily subjected to pre-treatment before conducting chemometric analysis to attenuate light scattering, baseline variations, and systematic noise. Pre-treatment represents a conventional procedure when developing identification and discrimination models through chemometric analysis in order to avert incorrect or negligible outcomes.[23] In this study, standard normal variate (SNV) was used as a pre-treatment method to remove the scatter effect.[12]

Principal Component Analysis (PCA)

This study used PCA, a robust statistical method, to show the variation and clustering in a dataset by lowering its dimensionality while preserving the most critical information.[24] The PCA biplots presented (Figure 2 A–D) show differences in the chemical composition of the various extracts (ethanol 96%, ethyl acetate, and n-hexane) from Auri and Mangium samples collected from different locations (BATOLA and BJB).

Each PCA plot shows the arrangement of the samples in a 2D space outlined by the first and second principal components. The divergence of the samples in these plots shows the chemical variations among the extracts, suggesting that different solvents extract has diverse compositions of bioactive compounds.

PCA biplot for 96% ethanol extract (Figure 2A) shows a distinct clustering pattern among the samples. This is because ethanol is a polar solvent, which predominantly extracts hydrophilic compounds such as flavonoids, phenolics, and certain glycosides.[25] The samples named "Auri BATOLA" and "Mangium BATOLA" seem to differ from "Auri BJB" and "Mangium BJB", indicating that geographical or environmental factors might affect their characteristics. Furthermore, the spread in each sample group indicate variations in the specific categories, potentially due to differences in chemical composition, or processing conditions.[26]

One crucial aspect of PCA is understanding how much variance is explained by each principal component (PC). In comparison, PC1 captures the most significant variance, while PC2 explains the next most important variations. Separation along PC1 indicates the potential to capture the main compositional differences in the compounds extracted from ethanol. This suggests that ethanol effectively differentiates samples based on their hydrophilic compound content.

Ethyl acetate is recognized as a semipolar solvent that extracts moderately polar substances like flavonoid aglycones, alkaloids, and certain terpenoids.[27] In this study, PCA plot pertaining to ethyl acetate extract (Figure 2B) showed a distinct clustering pattern. The groups Auri BATOLA and Mangium BATOLA were more dispersed, suggesting higher variability in their compositions. However, BJB group was more closely clustered, indicating similar compound profiles among these samples. PC1 still accounted for the main distinction between the extracts.

PCA plot for n-hexane extract (Figure 2C) shows that n-hexane, a nonpolar solvent, is predominantly used to extract lipophilic substances

like essential oils, fatty acids, and nonpolar terpenoids.[28] In line with the results, there was a distinct separation between BATOLA and BJB samples, showing variations in lipophilic profiles based on location. The clustering was more dispersed compared to ethanol and ethyl acetate extracts, which implied greater chemical variation in nonpolar compounds. Some sample groups overlapped, possibly due to shared nonpolar metabolites. These results affirm that n-hexane primarily extracts hydrophobic compounds, leading to clustering distinct from polar and semipolar extracts.

Figure 2D shows a combined PCA analysis comprising all extraction solvents, including 96% ethanol, ethyl acetate, and n-hexane. The results showed three distinct clusters corresponding to solvent extractions, indicating the critical influence of solvent polarity on metabolite extraction. Ethanol extracts (green) occupied a separate

position from ethyl acetate (blue) and n-hexane extracts (red), showing the differences in extracting hydrophilic versus lipophilic compounds. This evident separation of extracts underscores that each solvent retrieves a unique subset of bioactive compounds, emphasizing the significance of solvent choice in chemical profiling.

PCA analysis offers important insights into the chemical diversity of Auri and mangium samples using various extraction solvents. The results correspond with known solvent polarity principles. Specifically, ethanol (polar) is effective in extracting phenolics, flavonoids, and glycosides, leading to unique clustering. Ethyl acetate (semi-polar) extracts moderately polar compounds with intermediate clustering behavior. n-Hexane (non-polar) acts as a lipophilic extract, leading to a distinctive clustering pattern.

The geographical factor (BATOLA versus BJB) influences the chemical composition, as observed in the distinct clustering in each extract type. These results show how well PCA can distinguish chemical compositions according to solvent polarity, which is crucial for focused extraction in the pharmaceutical, nutraceutical, and food sectors.

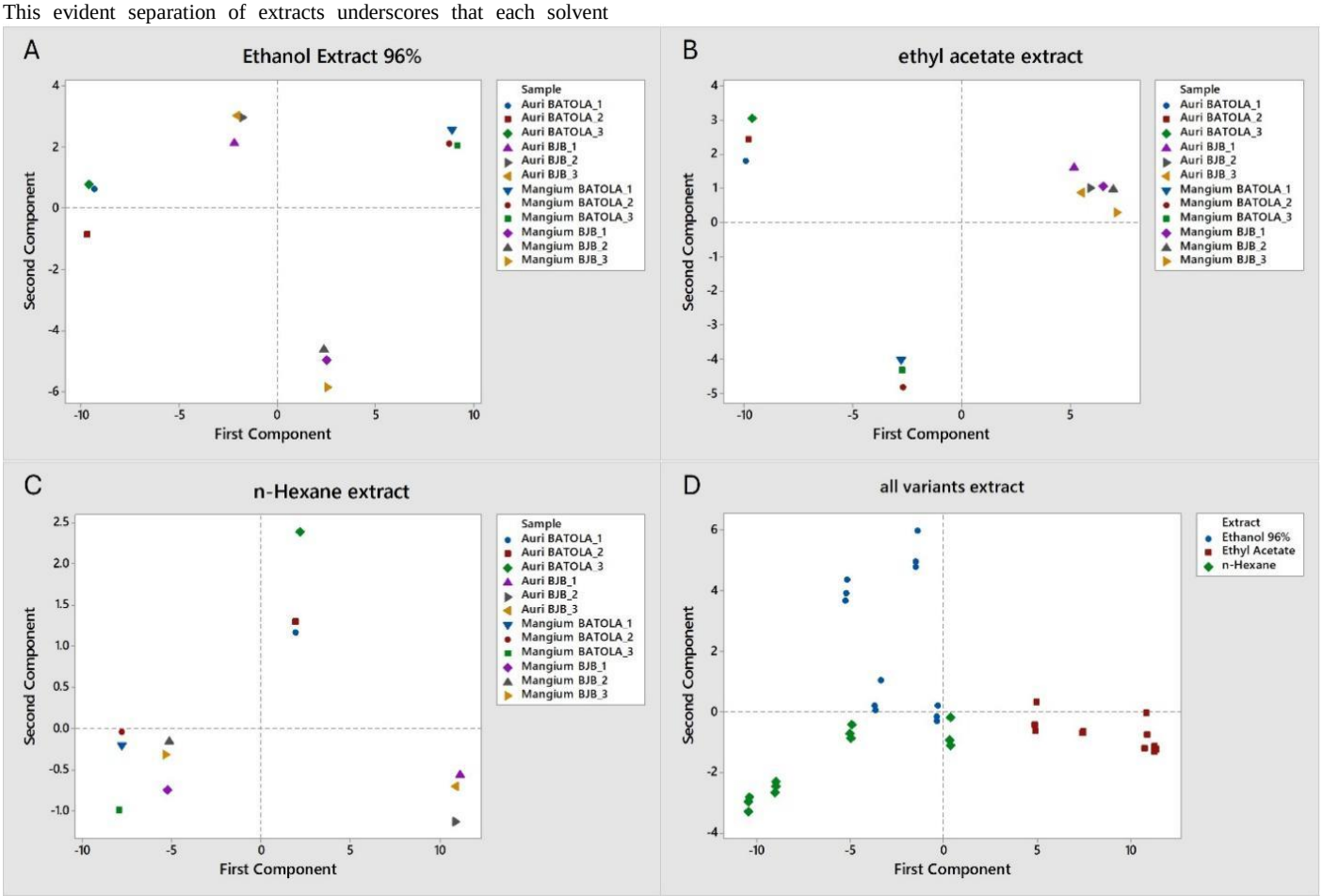

Figure 2: Principal component analysis (PCA) plot of samples: **A.** ethanol (96%) Extract, **B.** ethyl acetate extract, **C.** n-hexane extract and **D.** all variants extract

Partial Least Square (PLS)

PLS regression plots shown in Figures 3A–D were developed to predict SPF values for two Acacia leaves of different origin with various extracts (96% ethanol, ethyl acetate, n-hexane, and a mixture of all extracts). In PLS plots, SPF serves as an essential indicator of photoprotective qualities, assessing a substance's ability to absorb or block UV radiation.[14] These plots allow the examination of the correlation between actual SPF values and predicted outcomes, assessing the model's precision and UV protection effectiveness of different solvent extracts. The actual SPF values (x-axis) are those measured from *in vitro* assays, as shown in Table 2. The calculated response (y-axis) represents SPF values predicted from UV-Vis spectroscopy directly by PLS model. A strong correlation (high R^2 and R^2 Pred) suggests that the extraction process and selected solvent effectively isolate UV-protective compounds.[22,23,26]

SPF prediction for the 96% ethanol extract (Figure 3A) showed R^2 of 0.999 and R^2 Pred of 0.971, using 7 components. Furthermore, there was a strong correlation between SPF values and model predictions. The minor variance between actual and predicted SPF shows that ethanol extracts consistently contain photoprotective compounds like flavonoids and polyphenols. The 96% ethanol serves as an excellent solvent for extracting UV-protective bioactives, supporting its widespread use in herbal sunscreen formulations.[29]

Figure 3B showed that SPF prediction for ethyl acetate extract had R^2 of 0.980 and R^2 Pred of 0.920 (incorporating 2 components). The result showed a strong correlation, but the value was slightly less compared to ethanol. Although ethyl acetate effectively extracts SPF-active substances, the lower R^2 Pred implies greater variability.[26] The model

showed slightly reduced stability, possibly related to the solvent's intermediate polarity. Ethyl acetate is effective in extracting SPF-enhancing compounds but may be lacking in broad-spectrum photoprotective elements compared to 96% ethanol.[29]
As shown in Figure 3C, the prediction of SPF for n-hexane extract was characterized by R^2 = 0.999 and R^2 Pred = 0.999, consisting of 5

components. The model showed a perfect fit without overfitting, indicating high reproducibility. The strong SPF prediction shows the significant contribution of nonpolar lipophilic compounds such as carotenoids and tocopherols to UV protection. n-hexane is highly effective in extracting UV-protective lipophilic compounds, underscoring its importance in formulations of oil-based sunscreens, including those based on vitamin E as UV protectants.
Figure 3D shows SPF prediction for all combined extracts with R^2 = 0.999; R^2 Pred = 0.997, consisting of 7 components. The model continues to be highly predictive when all extracts are combined. This correlation indicates the presence of SPF-active compounds in all solvent extracts. A multisolvent extraction method can also offer comprehensive UV protection by integrating polar, semipolar, and nonpolar bioactives for better photoprotection.

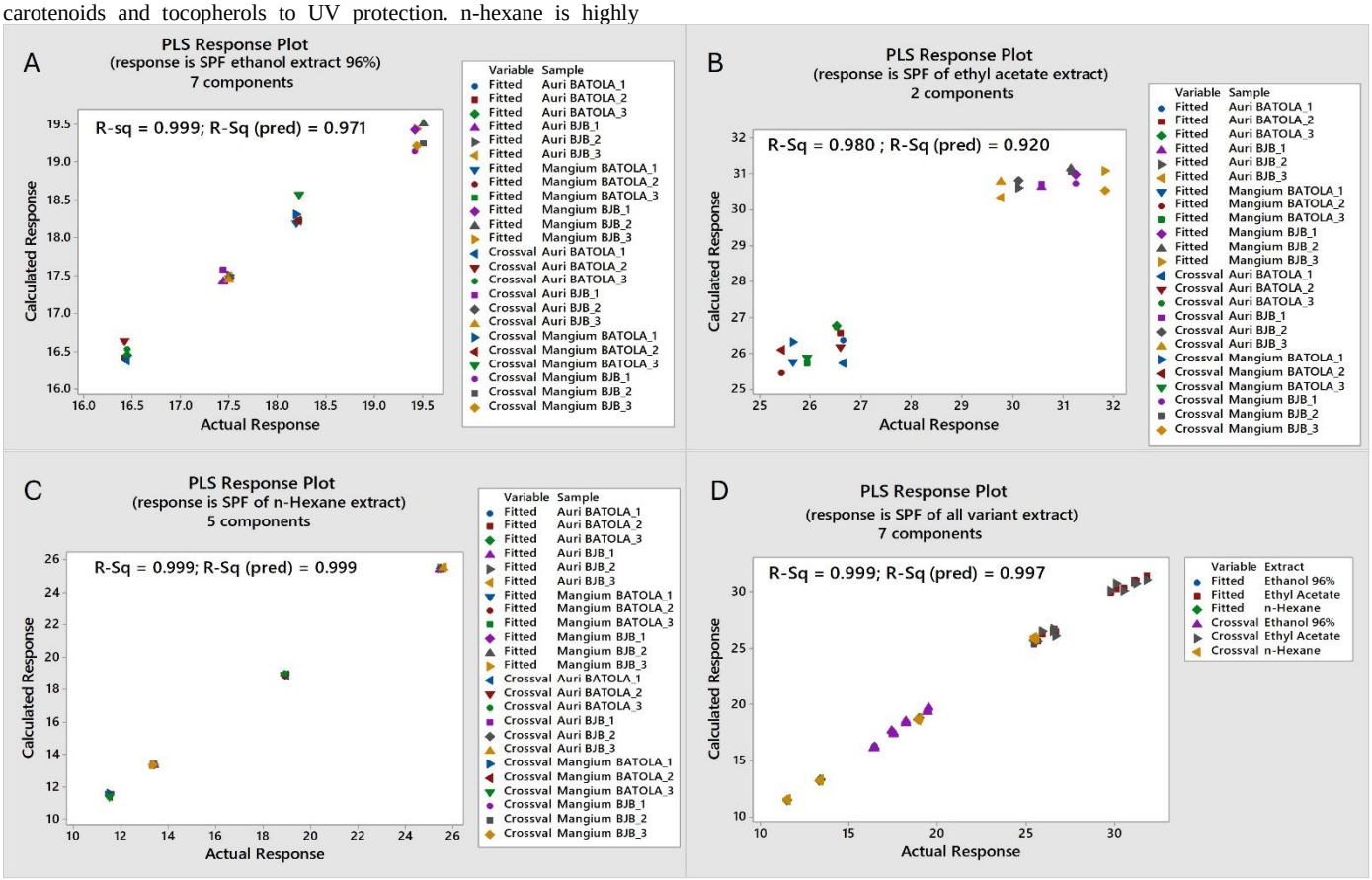

Figure 3: Partial Least Square (PLS) plot of samples: **A.** ethanol (96%) extract, **B.** ethyl acetate extract, **C.** n-hexane extract, and **D.** all variants extract

The results indicate that UV-Vis spectroscopy, when integrated with PLS regression analysis, influence SPF-active compound extraction by different solvents. Minimal overfitting and high cross-validation accuracy confirm the reliability of these models. It is effective in accurately determining SPF value in ethanol, ethyl acetate, and n-hexane extracts of two species of Acacia leaves in a short period, eliminating the requirement for sample preparation.

Conclusion

In conclusion, the effectiveness of UV-Vis spectroscopy combined with chemometric analysis proved effective in predicting SPF of Acacia extracts. The results showed that solvent polarity, plant species, and geographical origin significantly influenced SPF values and the extraction of UV-absorbing bioactive compounds. PCA successfully differentiated extracts, and PLS regression (R^2 > 0.99) accurately predicted SPF values, thereby validating the reliability of chemometric models for SPF estimation. This study also enabled rapid, accurate, and non-invasive SPF evaluation of plant-based sunscreen ingredients by integrating UV-Vis spectroscopy and chemometric models, which supported the development of sustainable and effective photoprotective solutions. The findings from this study have revealed Acacia extracts as a potential natural sunscreen agents, with ethyl acetate and n-hexane extracts having the highest SPF values. Moreover, future studies should be conducted, focusing on compound identification (LC-MS), *in vivo* SPF validation, and development of sunscreen formulation to enhance natural, eco-friendly photoprotection strategies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The authors are grateful for the financial support from LPPM, Lambung Mangkurat University. The authors also express their gratitude to Dr. Gunawan for the identification of Acacia leaves and Laboratorium Terpadu ULM for the collaboration.

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