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KI PAHIT (TITHONIA DIVERSIFOLIA) AS A SUSTAINABLE PLANT-BASED SUN PROTECTION SOURCE: A PHYTOCHEMICAL APPROACH

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K E Y W O R D

Phytochemical;
SPF;
Tithonia Diversifolia.

A B S T R A C T

Phytochemical compounds are bioactive substances found in plants that can be utilized as herbal medicine, antioxidants, and natural sun protection agents. Sun Protection Factor (SPF) is defined as the ratio between the amount of solar energy required to produce minimal erythema on protected skin and that required on unprotected skin. This study aimed to analyze the phytochemical compounds present in ki pahit (Tithonia diversifolia) leaves and evaluate their potential as a natural sun protection source. This research was conducted as an in-vitro experimental study in the laboratory using ki pahit leaf extract as the testing material. Phytochemical analysis was performed using the GC-MS (Gas Chromatography–Mass Spectrometry) method, while SPF analysis was carried out in-vitro using a UV-Vis spectrophotometer. The phytochemical analysis revealed that ki pahit leaf extract contained six major active compounds with the highest quality values, namely benzyl alcohol, p-hydroxy-alpha-[(methylamino) methyl]; 1- [alpha- (1-adamantyl) benzylidene] thiosemicarbazide; hexadecanoic acid (CAS); phytol isomer; benzenemethanol, 2-(2-aminopropoxy)-3-methyl-; and 7,9-dihydroxy-5-methoxy-2-methyl-1,4-anthracenedione. The SPF test was arranged using a Completely Randomized Design (CRD) with five treatment concentrations, namely 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm, with three replications for each treatment. The results of SPF analysis showed significant differences among concentrations. The highest SPF value was obtained at the concentration of 250 ppm with an SPF value of 7.17, which was categorized as extra protection.

INTRODUCTION

Free radicals cause cellular and tissue damage due to the presence of reactive oxygen species (ROS), which adversely affect essential biological components such as DNA, lipids, carbohydrates, and proteins (Susantiningsih, 2021). One of the most common causes of skin inflammation is exposure to ultraviolet (UV) radiation. High-intensity UV exposure can induce various skin disorders, ranging from mild irritation, burning sensations (sunburn), erythema (skin redness), hyperpigmentation, and even skin cancer (Adzhani *et al*., 2022). One of the preventive measures against the harmful effects of UV radiation is the application of sunscreen products containing antioxidant compounds (Jesus et al., 2023).

The protective efficacy of sunscreen products is determined by their Sun Protection Factor (SPF) value. SPF is defined as the ratio between the amount of solar energy required to induce minimal erythema on sunscreen-protected skin and that required on unprotected skin (Nopiyanti & Aisiyah, 2020). A higher SPF value indicates greater effectiveness in protecting the skin from UV radiation (Melitia *et al*., 2023). According to the Indonesian Food and Drug Authority (BPOM), sunscreen products are classified based on their SPF

values: SPF ≥6–<15 is categorized as low protection, SPF ≥15–<30 as moderate protection, SPF ≥30–<50 as high protection, and SPF ≥50 as very high protection. The SPF value is strongly influenced by the concentration of antioxidant compounds present in the product. Antioxidants are capable of interrupting free radical chain oxidation reactions by inhibiting the excessive accumulation of oxidizing agents within the body (Handajani, 2019).

Secondary metabolites are naturally occurring bioactive compounds synthesized by plants and serve as important sources of medicinal substances (Kurniawati *et al*., 2020). One tropical plant widely utilized in traditional medicine is Tree Marigold (*Tithonia diversifolia*). This species is widely distributed throughout Indonesia and is locally known as *Ki Pahit*. *T. diversifolia* possesses numerous pharmacological properties due to the presence of various secondary metabolites, including flavonoids, alkaloids, steroids, and other bioactive compounds. Phytochemical screening and Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the aqueous leaf extract of *T. diversifolia* revealed the presence of flavonoids, alkaloids, tannins, and twelve additional chemical constituents (Amanatie & Eddy Sulistyowati, 2015). The phytochemical composition of *T. diversifolia* can be determined through phytochemical analysis, among which Gas Chromatography–Mass Spectrometry (GC–MS) is one of the most widely employed techniques. GC–MS combines gas chromatography (GC) for the separation of volatile compounds with mass spectrometry (MS) for compound identification. Gas chromatography functions as a separation system for complex mixtures present in a sample, whereas mass spectrometry serves to detect and identify the individual compounds separated during the chromatographic process (Nurhaen *et al*., 2016).

METHOD

Materials

This study was conducted at the Genetic Resources and Molecular Biology Laboratory, Udayana University, Bali, Indonesia. The materials used in this study included *Tithonia diversifolia* (Ki Pahit) leaves, 96% ethanol as the extraction solvent, distilled water (aquadest), 70% alcohol, and a blank solution.

Methods

This research was an in vitro experimental study conducted under laboratory conditions. The bioassays performed included phytochemical analysis and Sun Protection Factor (SPF) evaluation. The initial stage involved extracting *T. diversifolia* leaves using 96% ethanol through maceration followed by solvent evaporation. The resulting leaf extract was subsequently used for the bioassays.

Phytochemical Analysis

Phytochemical analysis was performed using Gas Chromatography–Mass Spectrometry (GC–MS) on *T. diversifolia* leaf extract. The operating principle of gas chromatography involves injecting a liquid sample into an injector where it is vaporized. The vaporized sample is then carried by a carrier gas through a chromatographic column where compound separation occurs. Compounds with higher boiling points generally exhibit longer retention times than compounds with lower boiling points.

The principle of mass spectrometry is based on bombarding the analyte molecules with an electron beam and quantitatively recording the resulting positively charged ion fragments as a mass spectrum. These positive ion fragments are subsequently separated according to their mass-to-charge ratio, enabling the determination of the molecular weights of the compounds present in the sample. The GC–MS analysis generates data describing the chemical characteristics and concentrations of the identified compounds.

Sun Protection Factor (SPF) Determination

The SPF value was determined in vitro by measuring absorbance using a UV–Visible spectrophotometer within the wavelength range of 290–320 nm at intervals of 5 nm. The tested samples consisted of *T. diversifolia* leaf extract at concentrations of 50, 100, 150, 200, and 250 ppm, with each treatment performed in triplicate.

The absorbance values obtained for each concentration were recorded and used to calculate the SPF value according to the following equation:

$$\mathbf{SPF = CF \times \sum EE\ (\lambda) \times I(\lambda) \times abs(\lambda)}$$

Where:

CF : Correction factor (10)

EE : Erythemal effect spectrum

I : Solar intensity spectrum

Abs : Sample absorbance

The effectiveness of sunscreen protection was classified according to SPF values based on Damogalad *et al*. (2013), as cited by Krisyanella and Meinisati (2022), as follows:

Table 1. Sunscreen Effectiveness Classification Based on SPF Values

SPF Value	Sunscreen Protection Category
2-4	Minimal Protection
4-6	Moderate Protection
6-8	Extra Protection
8-15	Maximum Protection
>15	Ultra-Protection

RESULTS

Phytochemical Analysis

The results of the phytochemical analysis of *Tithonia diversifolia* (Ki Pahit) leaf extract using Gas Chromatography–Mass Spectrometry (GC–MS) revealed the presence of 28 chemical compounds. The identified compounds are presented in Table 2.

Table 2. Phytochemical Compounds Identified in *Tithonia diversifolia* Leaf Extract by GC–MS Analysis

No	Compound Name	Molecular Formula	RT	Area (%)	Quality
1	Pirolidin, 2-dodecyl-1-methyl-	C15H31N	6.265	0.87	58
2	1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide	C14H23N3O2	8.567	0.24	60
3	Benzyl alcohol, p-hydroxy-.alpha.-[(methylamino)methyl]	C9H13NO2	8.690	0.33	72
4	L-alanine-4-nitroanilide hydrochloride	C9H11N3O3	9.234	0.35	43
5	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	C6H7N3O2	11.407	0.29	58
6	1-[alpha.-(1-adamantyl)benzylidene] thiosemicarbazide	C18H23N3S	14.801	0.27	64
7	Hexadecanoic acid (CAS)	C16H32O2	14.934	1.19	97
8	(Tetrahydroxycyclopentadienone) tricarbonyliron(0)	C8H4FeO8	15.401	0.23	58
9	Phytol isomer	C20H40O	16.556	0.91	94
10	2,4(1H,3H)-Pyrimidinedione, 5-nitro-	C4H3N3O4	16.738	0.76	49
11	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	C11H17NO2	16.986	0.49	62
12	7,9-Dihydroxy-5-methoxy-2-methyl-1 ,4-anthracenedione	C16H12O5	17.645	1.43	62
13	1-(4,5-Diphenyl-oxazol-2-yl)-ethylamine	C17H16N2O	17.875	1.97	42
14	7,9-Dihydroxy-5-methoxy-2-methyl-1 ,4-anthracenedione	C16H12O5	18.162	2.30	62

15	3,4-Heptadien-2-on, 3,5-dicyclopentyl-6-methyl-	C18H28O	18.587	5.19	56
16	(trans)-2-Azidocyclopentan-1-ol	C5H9N3O	18.775	3.27	55
17	1-Naphthalenamine, 4-nitro-	C10H8N2O2	18.971	6.17	56
18	9(2',2'dimethylpropanoilhydrazn)-3,6-dichloro-2,7bis[2(diethylamino)-	C30H42Cl2N4O3	19.106	1.92	46
19	3-[o-Methoxyphenyl]-5,6-[(1',2'-cyclohexyl)dihydro]thiazolo[2,3-c]-s -triazole		19.247	6.40	50
20	1-(3-Isobutyryl-bicyclo[1.1.1]pent -1-yl)-2-methylpropan-1-on	C13H20O2	19.438	11.05	38
21	2,5-Dimethyloxazole-4-carboxylic acid	C6H7NO3	19.520	20.16	46
22	(Tetrahydroxycyclopentadienone) tricarbonyliron(0)	C8H4FeO8	19.746	4.78	50
23	(Tetrahydroxycyclopentadienone) tricarbonyliron(0)	C8H4FeO8	19.972	5.35	45
24	(-)-Tomoxetine	C17H21NO	20.091	6.50	42
25	Dl-.alpha.-(methylaminomethyl)benzyl alcohol	C9H13NO	20.552	1.61	38
26	1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-	C9H13NO3	20.910	1.03	42
27	3,5-dibromo-4-hydroxy-6-methylpicolinic acid	C7H5Br2NO3	23.108	13.60	38
28	Benzyl alcohol, alpha.-(1-aminoethyl)m-hydroxy-	C9H13NO2	24.758	0.88	41

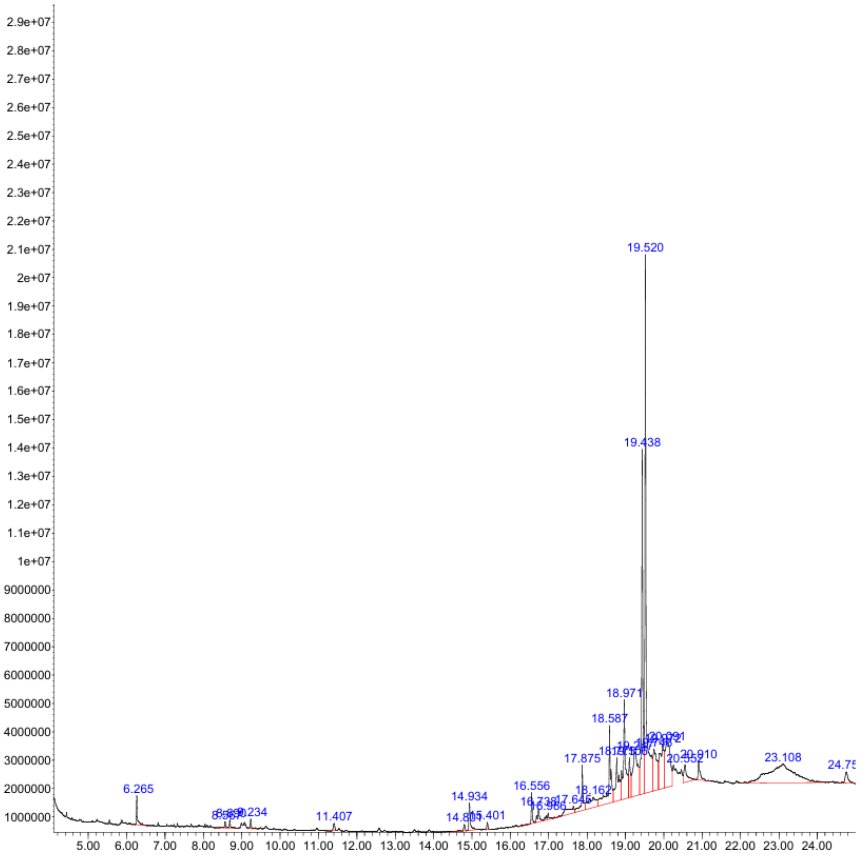

Figure 1. Gas Chromatography–Mass Spectrometry (GC–MS) Chromatogram Showing the Chemical Constituents Detected in *Tithonia diversifolia* Leaf Extract

Sun Protection Factor (SPF) Analysis

The SPF test results of *Tithonia diversifolia* (Ki Pahit) leaf extract, based on the analysis of variance (ANOVA), indicated that extract concentration had a significant effect on SPF values. The SPF values obtained at different extract concentrations are presented in Table 3.

Table 3. SPF Values of *Tithonia diversifolia* Leaf Extract

Concentration (ppm)	SPF Value	Protection Category
50	1,33a	Minimal Protection
100	2,85b	Minimal Protection
150	4,23c	Moderate Protection
200	5,85d	Moderate Protection
250	7,17e	Extra Protection

Note: Values followed by the same letter within the same column are not significantly different according to the Least Significant Difference (LSD) test at the 5% significance level.

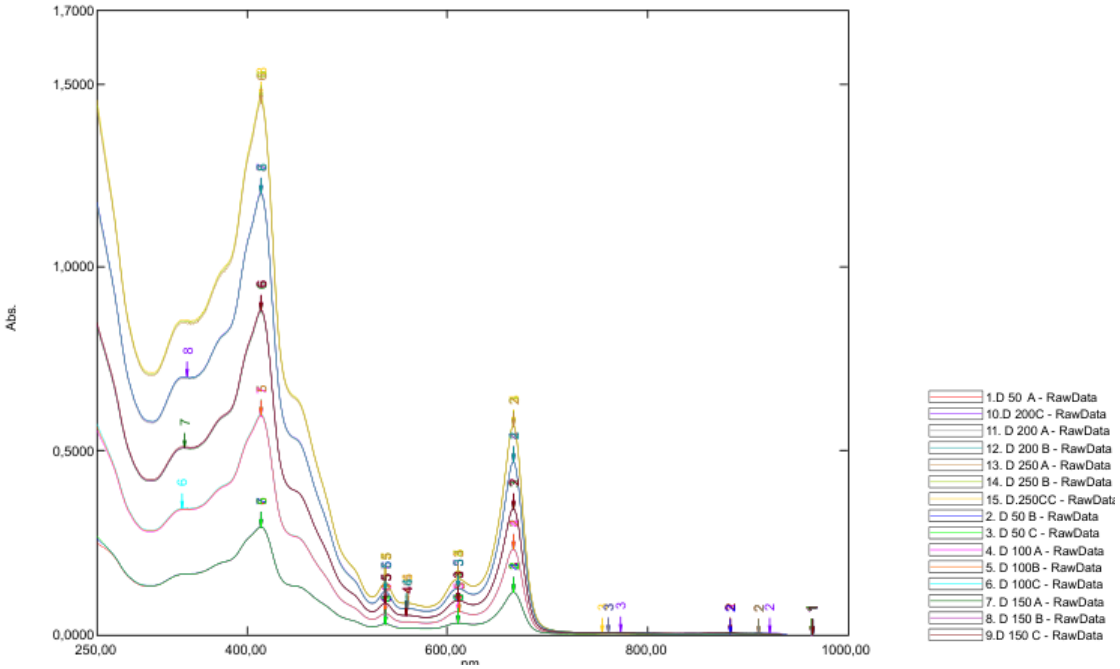

Figure 2. UV–Vis Spectrophotometric Determination of SPF Values in *Tithonia diversifolia* Leaf Extract

DISCUSSION

Based on the GC–MS phytochemical analysis of *Tithonia diversifolia* (Ki Pahit) leaf extract, six major bioactive compounds with the highest quality scores were identified, namely benzyl alcohol, *p*-hydroxy-α-[(methylamino)methyl]; 1-[α-(1-adamantyl)benzylidene] thiosemicarbazide; hexadecanoic acid (CAS); phytol isomer; benzenemethanol, 2-(2-aminopropoxy)-3-methyl-; and 7,9-dihydroxy-5-methoxy-2-methyl-1,4-anthracenedione.

The compound benzyl alcohol, *p*-hydroxy-α-[(methylamino)methyl], with the molecular formula $C_9H_{13}NO_2$, was detected with a quality score of 72 and a retention time (RT) of 8.690 min. This compound is commonly known as synephrine (*p*-synephrine), a phenethylamine alkaloid. Previous studies have reported that synephrine exhibits various biological activities and has been utilized as an antioxidant, antibacterial agent, cosmetic ingredient, insomnia treatment, and decongestant.

The bioactive compound 1-[α-(1-adamantyl)benzylidene] thiosemicarbazide, with the molecular formula $C_{18}H_{23}N_3S$, exhibited a quality score of 64 and an RT of 14.801 min. Several studies have demonstrated that this compound possesses antiviral and antioxidant activities. Consequently, it has been extensively investigated for pharmaceutical applications against viral infections and radiation-induced damage.

Hexadecanoic acid (CAS), with the molecular formula $C_{16}H_{32}O_2$, was identified with a quality score of 97 and an RT of 14.934 min. This compound, commonly known as palmitic acid, belongs to the class of saturated fatty acids. Hexadecanoic acid has been reported to exhibit various biological activities, including antibacterial, antifungal, and antioxidant properties. Shaaban et al. (2021) reported its antibacterial and antifungal activities, while Rajeswari *et al*. (2015) demonstrated its antioxidant potential.

Phytol isomer, with the molecular formula $C_{20}H_{40}O$, was detected with a quality score of 94 and an RT of 16.556 min. Phytol is a constituent of chlorophyll molecules and is synthesized by nearly all photosynthetic organisms, including higher plants, algae, and bacteria (de Souza & Nes, 1969; Ischebeck *et al*., 2006; Proteau, 1998, as cited in Islam *et al*., 2018). Phytol belongs to the terpenoid class of secondary metabolites (Hodiyah *et al*., 2019) and has been reported to possess antimicrobial and antioxidant activities.

The compound benzenemethanol, 2-(2-aminopropoxy)-3-methyl-, with the molecular formula $C_{11}H_{17}NO_2$, was identified with a quality score of 62 and an RT of 16.986 min. This compound is more commonly known as mexiletine. Mexiletine has been widely used in the treatment of neurological disorders, including myotonic syndromes (Conte *et al*., 2007), and has also been reported to alleviate neuropathic pain (Challapalli *et al*., 2005).

The SPF analysis revealed significant differences among the tested extract concentrations. The highest SPF value (7.17) was obtained at a concentration of 250 ppm, which was classified as providing extra protection. The antioxidant compounds present in *T. diversifolia* leaf extract may contribute to its photoprotective activity, thereby enhancing protection against ultraviolet (UV) radiation. Antioxidants are known to mitigate the harmful effects of UV-induced oxidative stress and may help prevent skin disorders associated with UV exposure, including irritation, erythema, and pigmentation resulting from excessive solar radiation (Mansur *et al*., 2016).

CONCLUSION

The findings of this study indicate that *Tithonia diversifolia* leaf extract contains several bioactive compounds, including flavonoids, alkaloids, terpenoids, and saturated fatty acids, which are known for their antioxidant activities. These compounds contribute to the extract's photoprotective potential, suggesting that *T. diversifolia* may serve as a promising natural source of sunscreen active ingredients.

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