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Original Research Article

GC-MS Analysis, Determination of Total Flavonoid Content, Sun Protection Factor, and *In Vitro* Anti-inflammatory Activity of Ethanol Extract of Kokang (*Lepisanthes amoena*) Leaves

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ABSTRACT

Lepisanthes amoena, locally known as kokang, is an indigenous plant from Kalimantan. The plant is traditionally used by the Dayak and Kutai tribes in East Kalimantan (Indonesia) for the treatment of skin inflammation. This study aimed to determine the phytochemical constituents, total flavonoid content, sun protecting factor (SPF), and anti-inflammatory activity of the ethanol extract of *L. amoena* leaves. Powdered *L. amoena* leaves were extracted by maceration in 96% ethanol. The chemical constituents of the extract were identified by gas chromatography-mass spectrometry (GC-MS). Total flavonoid content was determined using colorimetric method. The determination of sun protecting factor (SPF) was carried out using a UV-Vis spectrophotometer at a wavelength range of 290-320 nm. Anti-inflammatory activity was tested using the protein denaturation inhibition assay. GC-MS analysis identified five key volatile constituents, with Benzyl (1,2,3-thiadiazol-4-yl) carbamate (29.37%) identified as the major compound. The total flavonoid content of ethanol extract of *L. amoena* leaves was 7.105 ± 0.065% w/w QE. Results of the SPF determination indicate that *L. amoena* leaf extract has "ultra" UV-protection activity at 850–950 ppm with SPF values ranging from 16.351 to 20.543. The ethanol extract of *L. amoena* leaves exhibited strong anti-inflammatory activity, with IC_{50} value of 18.933 ppm in the protein denaturation inhibition assay, which was significantly lower than that of diclofenac sodium (IC_{50} = 23.006 ppm). These findings suggest that *L. amoena* leaf extract holds potential as a natural source of anti-inflammatory and photoprotective agents.

Keywords: *Lepisanthes amoena*, Gas Chromatography-Mass Spectrometry, Total flavonoids, Anti-inflammatory, Sun protecting factor.

Introduction

Lepisanthes amoena (Hassk.) Leenh., commonly known as kokang, is a medicinal plant traditionally used by the Dayak and Kutai tribes in East Kalimantan for the treatment of skin inflammation. Empirical use of kokang leaves include topical application as a traditional herbal cooling powder to manage skin conditions like acne.[1] Phytochemical investigations have revealed that *L. amoena* leaves contain various secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, saponins, and steroids.[2] Plants in the Sapindaceae family also exhibit relevant pharmacological benefits, supporting the potential of *L. amoena* as a therapeutic candidate.[3] Among these secondary metabolites, flavonoids are particularly noteworthy for their anti-inflammatory properties. Their mechanism of action involves the inhibition of protein denaturation through interaction with albumin molecules. The hydroxyl groups and aromatic rings present in flavonoids contribute to their ability to stabilize protein conformation, thereby preventing structural degradation and associated inflammatory responses.[4] Computational evidence from natural products confirms flavonoid-related mechanisms acting through modulation of iNOS and NF-κB inflammatory pathways. [5]Acne is a common dermatological disorder characterized by bacterial colonization and inflammation of the pilosebaceous units.[6] Inflammation is a protective physiological response to harmful stimuli, but chronic or excessive inflammation can result in tissue damage. Current pharmacological treatments for inflammation include steroidal anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs), both of which are associated with significant side effects when used long-term.[7] Therefore, alternative therapeutic agents from natural sources are being explored. The protein denaturation inhibition assay is an effective *in vitro* method to evaluate anti-inflammatory activity. It assesses a compound's ability to protect protein structure against denaturing agents. This assay is typically conducted using UV-Visible spectrophotometry.[8] As protein denaturation is closely linked to inflammatory mechanisms, inhibitors of this process are considered promising anti-inflammatory candidates.

Given the traditional use of *L. amoena* and its flavonoid content, this study aims to determine the total flavonoid content and evaluate the anti-inflammatory activity of the ethanol extract of *L. amoena* leaves using the protein denaturation inhibition method. To the best of our knowledge, no peer-reviewed journal articles have reported the anti-inflammatory activity of *L. amoena* leaves using the heat-induced protein denaturation inhibition assay, particularly in combination with GC–MS profiling, total flavonoid quantification, and in vitro sun protection factor (SPF) evaluation. The findings from this research are expected to contribute valuable preliminary data for the further pharmacological development of *L. amoena* as a natural anti-inflammatory agent.

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Materials and Methods

Chemicals and reagents

The chemicals and reagents used in this study include distilled water, aluminum chloride (Merck, Germany), glacial acetic acid (Merck, Germany), bovine serum albumin (HiMedia, India), 96% ethanol (Merck, Germany), methanol (Merck, Germany), sodium chloride (Xilong Scientific Co., China), diclofenac sodium (Aarti Drugs Ltd, India), quercetin (Sigma-Aldrich, USA), and tris base (Merck, Germany).

Plant collection and identification

Fresh leaves of *Lepisanthes amoena* were collected in August 2023 from Loh Pari Village, Tenggarong District, Kutai Kartanegara Regency, East Kalimantan, Indonesia (GPS coordinates: 0°22′40.80″S, 117°00′22.18″E). The plant material was authenticated at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, and voucher specimen no. 253a/LB.LABDASAR/IX/2023 was deposited for future reference.

Preparation of plant extract

A total of 250 g of dried powdered *L. amoena* leaves were subjected to maceration (in quadruplicate) with 96% ethanol (2.5 L). The solvent was added until it covered the powdered sample by approximately 2 cm. The extraction process lasted for 7 days, with solvent renewal every 24 hours and intermittent stirring every 8 hours. The setup was kept at room temperature and protected from light using aluminum foil. After each 24-hour period, the extract was filtered, and the filtrate was pooled. The combined extract was concentrated using a rotary evaporator at 50°C, followed by further evaporation on a water bath to yield a semisolid crude extract. The extract was weighed repeatedly at 1-hour intervals until a constant weight was achieved.[9]

GC-MS analysis

The GC-MS analysis was performed using an Agilent 8890 Gas Chromatograph coupled with an MS5977B Mass Selective Detector. A sample volume of 1 µL was injected in split mode with a split ratio of 50:1. Separation was carried out on an HP-5MS UI capillary column (30 m × 0.25 mm i.d, film thickness 0.25 µm). Helium was employed as the carrier gas at a constant flow rate of 1 mL/min and a column head pressure of 7.6522 psi. The oven temperature was initially programmed at 50°C and held for 2 min, then increased at a rate of 10°C/min to 200°C and maintained for 5 min. The injector temperature was set at 250°C throughout the analysis. The MS transfer line was maintained at 200ºC. The ionization mode used was electron ionization at 70eV. Compound identification was assessed using Total Ion Count (TIC). The spectra of the separated compounds were compared with the database of the NIST Reference Spectra Library. The relative percentage composition of the identified compounds was estimated from the GC peak area percentage.

Determination of total flavonoid content

A total of 100 mg sample of the ethanol extract was dissolved in absolute ethanol and transferred to a 10 mL volumetric flask, then diluted to volume with ethanol to obtain a stock solution of 10,000 ppm. From this, 1 mL was diluted to 10 mL to produce a 1,000 ppm working solution. One milliliter of this solution was mixed with 1 mL $AlCl_3$ solution and 8 mL of 5% acetic acid, vortexed, and allowed to stand at room temperature. The absorbance was measured at the predetermined maximum wavelength using a UV-Vis spectrophotometer (PerkinElmer Lambda 360, USA). Quercetin (20 – 100 ppm) was used as the reference standard. The total flavonoid content was estimated from the equation of the quercetin standard curve, and expressed as mg quercetin equivalent per gram (mg QE/g).[10]

Determination of sun protection factor (SPF) evaluation

A total of 1000 ppm stock solution of the extract was prepared in 95% ethanol. From this stock, varying volumes (3 mL, 4 mL, 5 mL, 6 mL, and 7 mL) were pipetted and diluted to 10 mL to obtain final concentrations of 750–950 ppm. The SPF was determined *in vitro* using a UV-Vis spectrophotometer, measuring absorbance at 290–320 nm (5 nm intervals), and calculating the average absorbance for each sample. The SPF value was calculated using the Mansur equation (equation 1):

$$SPF = CF \times \sum EE(\lambda) \times I(\lambda) \times Abs(\lambda). \quad (1)$$

Where; CF is the correction factor, set at 10; EE(λ) represents the erythemal effect spectrum; I(λ) denote the solar intensity spectrum simulated under laboratory conditions; Abs(λ) refers to the absorbance of the formulation at the specified wavelength.[11]

Anti-inflammatory activity assay

The anti-inflammatory activity was assessed via the protein denaturation inhibition method. Ethanol extract and diclofenac sodium (positive control) were each dissolved in methanol p.a. to obtain 2000 ppm stock solutions. Serial dilutions (62.5, 125, 250, 500, and 1000 ppm) were prepared. From each dilution, 0.5 mL was mixed with 0.2% BSA in a 5 mL volumetric flask to yield final concentrations of 6.25 - 100 ppm. Samples were incubated at 25°C for 30 minutes, then heated at 70°C for 5 minutes and cooled at room temperature for 10 minutes. Absorbance was measured at 660 nm.[12,13] The inhibition of protein denaturation was calculated using the formula (equation 2):

$$\% \text{ inhibition} = \frac{ANC - ATU}{ANC} \times 100\%. \quad (2)$$

Where;

ANC = Absorbance of Negative Control, ATU = Absorbance of Test Solution

Samples showing >20% inhibition were considered to exhibit potential anti-inflammatory activity.[14]

Statistical analysis

Data were presented as mean ± standard deviation (SD), n = 3. IC_{50} value which represents the concentration of the sample required to inhibit inflammation by 50% was obtained using probit regression analysis performed with IBM SPSS Statistics version 26. Data were subjected to one-way analysis of variance, and differences between mean values were regarded as significant at $P < 0.05$.

Results and Discussion

Extraction and yield

The powdered *L. amoena* leaves exhibited a green colour, a characteristic leafy aroma, and a bitter taste, consistent with typical features of dried plant material. Extraction with ethanol resulted in a dark green, thick extract with a strong leafy aroma and a very bitter taste. The weight loss on drying was less than 0.5 mg (0.25%), and thus met the constant weight criteria.[7] The extract yield of 14.795 ± 0.059% (Table 1) aligns with a previous report, which indicated a yield of 15.9%,[13] though slightly lower than other studies which reported yields of 23% and 20.25%.[1,16]

Table 1: Extraction yield of *Lepisanthes amoena* leaves

Replicate	Weight of Powder (g)	Weight of Extract (g)	Yield (%)	Mean ± SD of Dry Extract Yield (%)	RSD (%)
1	250	37.190	14.876%	14.795 ± 0.059	1.969
2	250	37.060	14.824%		
3	250	36.890	14.756%		
4	250	36.810	14.724%		

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Chemical composition of L. amoena leaf extract

Analysis of the ethanol extract from kokang leaves (*Lepisanthes amoena*) using GC-MS has revealed the presence of several key bioactive constituents (Figure 1 & Table 2), indicating notable pharmacological potential. The most abundant compound identified was Benzyl (1,2,3-thiadiaze-4-yl)carbamate (8.56%), a derivative of thiadiazole carbamate, which is recognized for its pronounced antimicrobial properties.[17,18] Another significant constituent was 3-Thiazolidinecarboxylic acid, 4-(Acetyloxy)-2-(1,1-dimethylethyl)-, phenylmethyl ester, 1-oxide (3.12%), classified as a thiazolidine derivative with documented antimicrobial, anti-inflammatory, and anticancer effects.[19,20] Additionally, a pyrazole derivative (3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl 6.5%) was detected, which is also associated with anti-inflammatory and antimicrobial activities.[21,22]

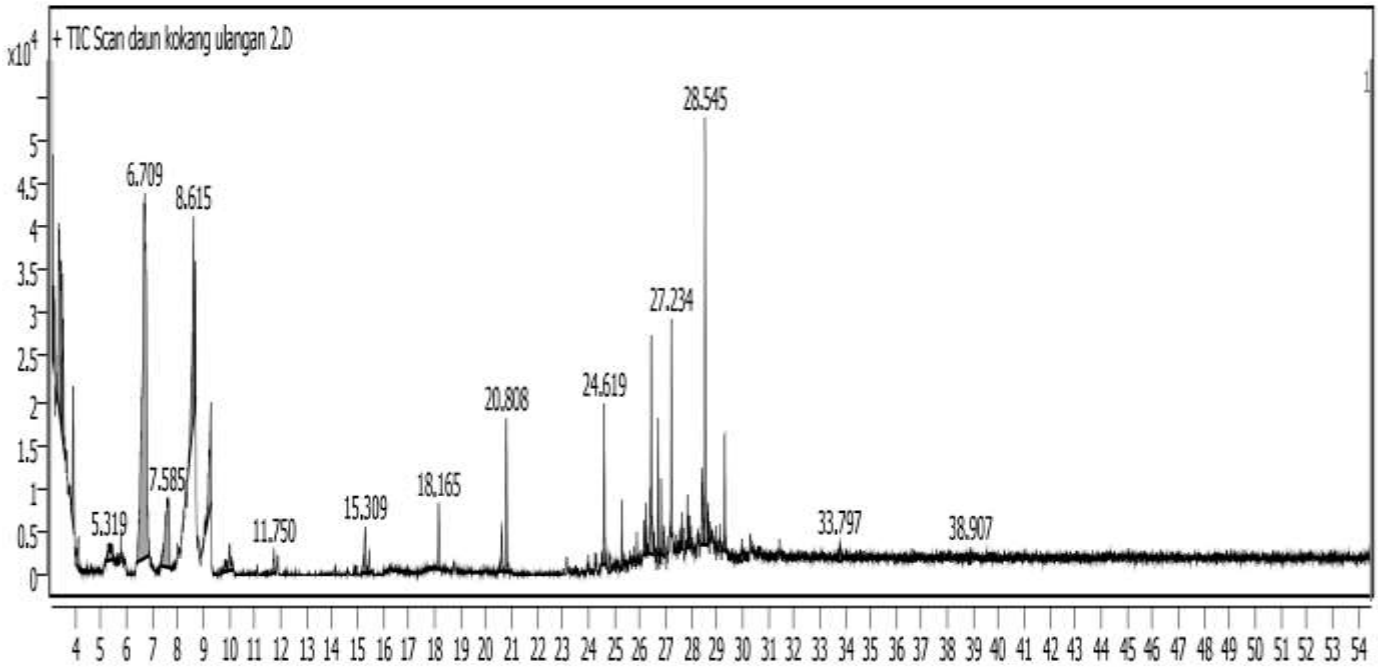

Figure 1: GC-MS chromatograms of ethanol extract of *Lepisanthes amoena* leaves

Table 2: Compounds identified from the GC-MS analysis of the ethanol extract of *Lepisanthes amoena* leaves

No	RT (min)	Compound name	Area %	Nature/ Description	Molecular Formula	Molecular Weight (g/mol)	Reference/ Activity
1	3.356	Benzyl (1,2,3-thiadiaze-4-yl) carbamate	8.56	Thiadiazole carbamate derivative	$C_{10}H_9N_3O_2S$	235.26	antimicrobial/antifung activity[17,18]
2	3.493	3-Thiazolidinecarboxylic acid, 4-(Acetyloxy)-2-(1,1-dimethylethyl)-,phenylmethyl ester, 1-oxide,[1R-(1.alpha.,2.beta.,4.beta.)]-	3.12	Thiazolidine derivative	$C_8H_{15}NO_4S$ (for tert-butyl ester, 1,1-dioxide)	221.27	antimicrobial, anti-flammatory, anticancer tivities[17,18]
3	8.615	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl	6.5	Pyrazole derivative	$C_{10}H_{16}N_2$	164.25	anti-inflammatory, antimicrobial activities[21,22]
4	8.683	Diazene, methylphenyl-1-oxide	2.1	Azo compound	$C_7H_8N_2O$	120,15	Antimicrobial[23,24]
5	9.278	Sydnone, 3-neopentyl	3.09	Heterocyclic mesoionic compound	$C_7H_{13}N_2O_2^+$	157.19	anti-inflammatory and timicrobial[25]

The presence of these compounds aligns with earlier findings that *L. amoena* leaves are rich in secondary metabolites such as saponins, flavonoids, and alkaloids, all of which contribute to their biological activities. For instance, research conducted by Rijai and colleagues (2024) demonstrated that the n-butanol fraction of *L. amoena* leaves contains a high concentration of saponins, which exhibited strong antioxidant and cytotoxic properties.[23] These saponins, as confirmed by LC-MS analysis, possess molecular weights exceeding 500 g/mol, a characteristic feature of biologically active saponins. Furthermore, the ethanol extract's content of polar compounds, including flavonoids and alkaloids, is believed to enhance its antimicrobial activity, as shown by inhibition zone tests against various pathogenic microbes.

Taken together, the GC-MS results reinforce the view that the ethanol extract of kokang leaves is a promising source of bioactive molecules with potential applications as antimicrobial, anti-inflammatory, and antioxidant agents, supporting their further development as phytopharmaceutical agents.

Total flavonoid content of L. amoena leaf extract

The total flavonoid content of the ethanol extract of *L. amoena* leaves was found to be 71.050 mg QE/g, equivalent to 7.105% w/w as quercetin equivalents. This value is relatively high, exceeding the flavonoid content of other plants within the Sapindaceae family, such as *Dimocarpus longan* (3.364% w/w QE),[12] and *Nephelium lappaceum* (2.339% w/w QE).[26] These findings highlight the potential of *L. amoena* as a promising natural source of flavonoids. The absorbance readings used to construct the quercetin standard curve are presented in Table 3, while the corresponding calibration graph is shown in Figure 1. Additionally, the total flavonoid content of the ethanol extract of *Lepisanthes amoena* leaves is summarized in Table 4.

UV protection activity and sun protecting factor of L. amoena leaf extract

UV protection activity testing showed that the ethanol extract of *L. amoena* leaves achieved a "maximum" protecting effect at concentrations of 750 and 800 ppm with SPF values of 14.705 and 14.584, respectively, and an "ultra" protection at 850, 900, and 950 ppm with SPF of 16.351, 17.981, and 20.543, respectively (Table 5). These results support the potential use of *L. amoena* extract as a natural sunscreen agent.

Table 3: Absorbance values of quercetin for construction of standard curve

Concentration (ppm)	Replicate	Absorbance	x̄ Absorbance	RSD (%)
	1	0.2045		
20	2	0.2036	0.204	0.221
	3	0.2040		
	1	0.3045		
40	2	0.3032	0.303	0.514
	3	0.3014		
	1	0.3906		
60	2	0.3886	0.389	0.264
	3	0.3892		
	1	0.4866		
80	2	0.4885	0.487	0.238
	3	0.4864		
	1	0.5779		
100	2	0.5787	0.578	0.147
	3	0.5796		

Table 4: Total flavonoid content of the ethanol extract of *Lepisanthes amoena* Leaves

Abs	Total Flavonoid Content (mg QE/g)	Average total flavonoid content (mg QE/g) ± SD	RSD (%)
0.4431	7.036	7.105 ± 0.065	0.918
0.4467	7.113		
0.4492	7.166		

Table 5: Sun protection factor (SPF) of ethanol extract of *Lepisanthes amoena* leaves

Concentration (ppm)	SPF	Category
750	14.705	max
800	14.584	max
850	16.351	ultra
900	17.981	ultra
950	20.543	ultra

Anti-inflammatory activity of L. amoena leaf extract

The ethanol extract of *L. amoena leaves* was tested for its ability to inhibit protein denaturation. All tested concentrations (6.25 – 100 ppm) resulted in inhibition values exceeding 20%, indicating anti-inflammatory activity.[14] Remarkably, the extract showed significant inhibition even at the lowest concentration (6.25 ppm), which was higher than that of the positive control (Diclofenac sodium).

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However, at higher concentrations (25, 50, and 100 ppm), the positive control demonstrated stronger inhibition compared to the extract. For instance, at 100 ppm, diclofenac sodium achieved 80.687% inhibition, while the *L. amoena* extract result in 69.640% inhibition (Table 6).
Probit analysis (SPSS version 26) yielded an IC_{50} value of 18.933 ppm for the ethanol extract of *L. amoena leaves* (Table 7). This result indicated a higher activity than the positive control (IC_{50} = 23.006 ppm). This finding indicates a higher anti-inflammatory activity for *L. amoena leaves* compared to findings from other studies, such as those on starfruit leaves (IC_{50} = 20.20 ppm)[27], moringa leaves (IC_{50} = 211.11 ppm)[10], and *Justicia secunda* reported by Anyasor (IC_{50} = 186.20 ppm).[28]

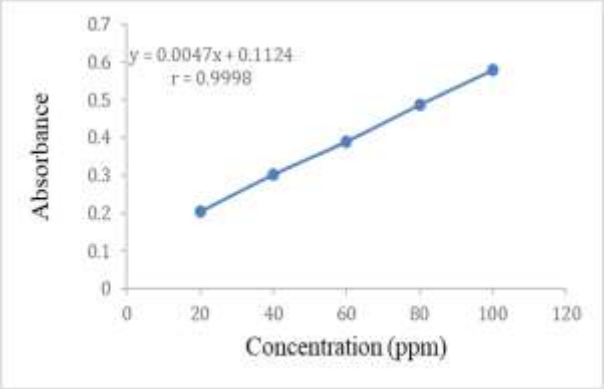

Figure 2: Quercetin standard curve for determination of total flavonoid content

Table 6: Percentage inhibition of protein denaturation by ethanol extract of *Lepisanthes amoena* leaves

Concentration (ppm)	% inhibition			Average % inhibition	SD	RSD (%)
	Rep 1	Rep 2	Rep 3			
6.25	38.280	37.830	38.106	38.072	0.227	0.596
12.50	44.779	44.994	44.955	44.910	0.115	0.256
25.00	52.185	52.186	52.327	52.233	0.081	0.156
50.00	60.965	60.602	60.549	60.705	0.226	0.372
100.00	69.854	69.541	69.526	69.640	0.185	0.266

Table 7: IC_{50} value of the ethanol extract of *Lepisanthes amoena* leaves based on protein denaturation inhibition assay

IC_{50} (ppm)	Mean IC_{50} ± SD (ppm)	RSD (%)
18.789	18.933 ± 0.144	0.758
19.076		
18.933		

As a reference, diclofenac sodium was tested at concentrations ranging from 6.25 to 100 ppm. Inhibition above 20% was observed at concentrations of 12.5 ppm and above, indicating strong anti-inflammatory activity.[11] However, at 6.25 ppm, the inhibition was below the 20% threshold (Tables 8 and 9).
The IC_{50} value obtained from probit analysis was 23.006 ppm. This value is lower than the results reported by Farida (2018)[29] and Fadlilaturrahmah (2022),[7] who reported IC_{50} values of 47.74 ppm and 48.14 ppm, respectively. Anyasor (2019) reported much higher IC_{50} of 215.50 ppm.[28] The relative standard deviation (RSD) of 0.839% indicates good precision and complied with the acceptable limit of ≤ 2%.[30]

Table 8: Percentage inhibition of protein denaturation by diclofenac sodium

Concentration (ppm)	% inhibition			Average % inhibition	SD	RSD (%)
	Rep 1	Rep 2	Rep 3			
6.25	19.424	18.749	19.059	19.077	0.338	1.771
12.50	39.232	38.890	38.866	38.996	0.204	0.524
25.00	52.551	52.453	52.663	52.556	0.105	0.200
50.00	67.535	67.281	67.401	67.406	0.127	0.188
100.00	80.832	80.566	80.663	80.687	0.135	0.167

Table 9: IC_{50} value of diclofenac sodium based on protein denaturation assay

IC_{50} (ppm)	Mean IC_{50} ± SD (ppm)	RSD (%)
22.805		
23.190	23.006 ± 0.193	0.839
23.024		

-

Conclusion

The GC-MS analysis of *L. amoena leaf extract identified five key compounds with promising biological activities. The* most abundant compound identified was Benzyl (1,2,3-thiadiazol-4-yl) carbamate, accounting for 8.56% of the total composition. The total flavonoid content was determined to be 71.050 mg QE/g, equivalent to 7.105% w/w of quercetin. The extract demonstrated notable potential as a natural sunscreen, particularly at concentrations ranging from 850 – 950 ppm, corresponding to the "ultra" protection category. Furthermore, the extract exhibited promising anti-inflammatory activity, with an average IC_{50} value of 18.933 ± 0.144 µg/mL, indicating a stronger activity than the positive control (Diclofenac sodium) with IC_{50} of 23.006 ± 0.193 ppm.

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.

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