CHEMISTRY & CHEMICAL TECHNOLOGY

Chem. Chem. Technol., 2025,
Vol. 19, No. 3, pp. 538–548

Chemical Technology

UNVEILING THE BIOACTIVE POTENTIAL OF KAFFIR LIME (*CITRUS HYSTRIX*) LEAVES: ANTIOXIDANT, ANTIMICROBIAL, AND PHOTOPROTECTIVE PROPERTIES

Bashini P. Fernando[1], Darshani H. D. DewageDona[1], Chandima S. K. Rajapakse[1,]*

https://doi.org/10.23939/chcht19.03.538

Abstract. Kaffir lime (*Citrus hystrix*), a citrus species native to Southeast Asia, has limited research concerning its pharmaceutical and cosmeceutical potential. This study investigates the antioxidant, antimicrobial, and photoprotective properties of the methanolic extract of Kaffir lime leaves. The results demonstrate that Kaffir lime leaves are a promising herbal source for pharmacological applications, as they are rich in phytochemicals with antioxidant potential, strong photoprotective properties with a high SPF value, and antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Aspergillus welwitschiae*.

Keywords: Antioxidant, Antimicrobial, Pharmacological, Photoprotective

1. Introduction

Citrus hystrix, commonly known as Kaffir lime, belongs to the genus Citrus within the Rutaceae family.[1] This tropical plant, typically found in Southeast Asia, particularly in Malaysia and Thailand, grows as a shrub or tree up to 12 meters in height.[2-3] The leaves are unifoliolate, broadly ovate to ovate-oblong, dark green on the upper side and light green underneath. In Sri Lanka, it is called Gada Dehi, Kudalu Dehi, or Leech lime.[4]

Kaffir lime has long been utilized in traditional medicine for treating various ailments, including hypertension, sore throat, digestive disorders, and fever. In Malaysia, its fruit pulp is used for body and hair care and as an insecticide for leeches.[2] The leaves are used to relieve abdominal pain caused by insect bites and potentially benefit dental health and scurvy prevention.[5] Additionally, the leaves are a popular ingredient in Thai cuisine and have insecticidal properties against mosquitoes and other pests. They also demonstrate antiviral activities, including inhibition of the Herpes virus, and antioxidant properties activity.[6]

The bark exhibits moderate antimicrobial properties, while the root extract has been shown to inhibit HIV-1 protease.[7] Kaffir lime's essential oil has potential applications in improving the qualities of bio-plastic materials derived from cassava peel waste.[8] The plant is recognized for its antioxidant, hepatoprotective, antibacterial, antifungal, and antileukemic activities.[6,9,10] The phytochemicals in Kaffir lime, including essential oils and phenolic compounds such as flavanones, are responsible for these bioactivities. Studies have identified major components of Kaffir lime essential oil, including citronellal, β-pinene, linalool, and limonene.[11-12] The chemical composition of these oils can vary depending on environmental factors. Leaf essential oils have demonstrated antimicrobial activity against various bacteria and fungi, including *Bacillus subtilis, Klebsiella pneumoniae, Edwardsiella tarda, Staphylococcus aureus, Escherichia coli, Salmonella paratyphi, Elizabethkingia meningoseptica, Staphylococcus epidermidis, Salmonella enteritidis, and Pseudomonas aeruginosa*, while methanolic extracts from the leaves have shown significant antioxidant capacities.[13]

Other than that, leaf essential oils inhibit the growth of *Aspergillus niger, Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes, Cryptococcus neoformans, Saccharomyces cerevisiae and Pyricularia oryzae* also.[14-15] Methanol extract of Kaffir lime leaves by Soxhlet extraction contained carbohydrates, flavonoids, glycosides, alkaloids, tannins, steroids and phenols. They showed good cupric-reducing antioxidant capacity, DPPH free radical scavenging activity, nitric oxide scavenging activity, and total antioxidant capacity. Alkaloids, steroids, flavonoids, tannins, saponins, and terpenoids were mainly detected from ethanolic extract of Kaffir lime plant parts extracted by the maceration technique. It exhibited inhibition against *Bacillus subtilis*.[5,16]

[1] Department of Chemistry, University of Kelaniya, Kelaniya, Sri Lanka.11600
[*] *shashikala@kln.ac.lk*

Although extensive research has been conducted on the various bioactivities of Kaffir lime leaves, there is a lack of studies specifically investigating their UV protection or photoprotective properties. Since it has been proven that Kaffir lime is abundant in various chemical compounds with bioactivities, investigation of novel bioactive molecules with pharmaceutical and cosmeceutical properties, particularly in their leaves will add value to the underutilized leaves and it will open new avenues of research and commercial scale cultivation, contributing to national development, science and technology capacity building in the country. Therefore, this study focuses on the extraction of bioactive compounds from the leaves of Kaffir lime grown in Sri Lanka and the investigation of the potential use of Kaffir lime leaves as a source of phytochemicals with antioxidant, antimicrobial, and photoprotective properties.

2. Experimental

2.1. Materials

Chemicals used for this study were methanol, dichloromethane, hexane, ethanol, acetone (Sigma Aldrich), glacial acetic acid, ethyl acetate, toluene, formic acid, aluminum chloride (Sigma Aldrich), butylated hydroxytoluene (Sisco Research Laboratories), Folin Ciocalteu phenol reagent (Thermo Scientific), Gallic acid (98%, S D Fine-Chem. Ltd, India), nutrient agar, nutrient broth, potato dextrose agar, dextrose, Quercetin (Thermo Scientific), silica gel (Sigma Aldrich), sodium acetate (AR/ACS grade, Finar), sodium carbonate (Sigma Aldrich), α-diphenyl-β-picrylhydrazyl (Aldrich), sodium nitrite (Sigma Aldrich), sodium hydroxide (Sigma Aldrich) and a commercial sunscreen product was used as the reference sunscreen.

2.2. Methods

2.2.1. Plant collection and pretreatment

Kaffir lime leaves were collected during the period from November - December 2021 from plant nurseries in Horana and Kurunegala, Sri Lanka. The collected leaves were washed and air-dried with mild sunlight for 6 days. Then, they were cut into small pieces and ground into coarse powder using a mechanical grinder. Finally, the powder was packed into an airtight bottle and stored at 4 ^{0}C until further analysis.

2.2.2. Extraction

Chemical constituents in Kaffir lime leaf powder (29.6 g) were extracted with methanol (350 mL) by maceration technique for 5 days. The extract was subjected to sequential fractionation by using hexane (50 mL × 2), dichloromethane (DCM) (100 mL), and methanol (50%, 100 mL). Each fraction was filtered and solvents were evaporated using a rotary evaporator at 37 ^{0}C (20 rpm) and dried. The obtained residues were weighed. Finally, they were kept at 4 ^{0}C until further analysis. The percent yield of each fraction was calculated using Eq. (1).

$$\text{Percentage yield} = \frac{weight\ of\ extract}{weight\ of\ dry\ leaves}\ x\ 100\% \quad (1)$$

2.2.3. Determination of antioxidant activity by α-diphenyl- β- picrylhydrazyl (DPPH) radical scavenging assay

DPPH (2,2-diphenyl-1-pycryl-hydrazil) radical scavenging activity was determined as described by Moragot Chatatikun and Anchalee Chiabchalard *et al.* with minor modification.[17] Stock solutions of extract/fraction and standard butylated hydroxytoluene (BHT) (1000 µg/mL) were prepared by using methanol. Then diluted to make final concentrations of 1000, 500, 250, 125, 62.2, 31.5, 15.62, and 7.81 µg/mL by using methanol. Methanol was used as the reagent blank. Samples, standard, and blank (160 µL) were added to the 96-well plate. Then methanolic DPPH solution (0.25 mg/mL, 40 µL) was added and mixed. The plate with samples was incubated for 30 min at room temperature in the dark. Finally, the absorbance of samples was measured at 517 nm with a microtiter plate reader (Thermo Fisher Scientific, USA). The radical scavenging activity was expressed as percent inhibition and was calculated by Eq. (2).

$$\text{Percentage inhibition} = \frac{A_{(control)} - A_{(sample)}}{A_{(control)}} \times 100\% \quad (2)$$

where $A_{(sample)}$ is the absorbance of the sample; $A_{(control)}$ is the absorbance of the control sample.

Obtained percentage inhibition values were plotted with the respective concentrations of the samples/standard to calculate IC_{50} values.

2.2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the fractions was determined by the Folin-Ciocalteu method described by Dewage *et al.* and Fettah Asma *et al.* with some modifications.[18-19] Concentration series of 100, 50, 25, 12.5, and 6.25 µg/mL of fractions (1000 µg/mL) and Gallic acid standard solution were prepared using distilled water. The blank was prepared by replacing the sample with distilled water. Samples/standard/blank (50 µL) and distilled water (50 µL) were added to a 96-well plate, respectively. Then Folin-Ciocalteu phenol reagent (10%, 50 µL) and sodium carbonate solution (1 M, 50 µL) were added to each solution in the 96-well plate. All reagents were allowed to stand in a dark at room temperature for 60 min. Finally, the absorbance of each sample was measured at 740 nm using a microplate reader (Thermo Fisher Scientific, USA). TPC was calculated by using a standard curve of Gallic acid. TPC was expressed as mg Gallic Acid Equivalents (GAE) per gram of dry plant material.

2.2.5. Determination of Total Flavonoid Content (TFC)

The total flavonoid content of each fraction was determined by the aluminum chloride colorimetric assay with slight modifications.[20] Samples (1000 µg/mL, 0.5 mL) were added to test tubes. The blank was prepared by replacing the sample with methanol. Quercetin was used as the standard solution. Then, 2.0 mL of distilled water followed by 150 µL of 5% $NaNO_2$ were added. Samples were incubated for 5 minutes at room temperature in a dark environment. Then 150 µL of 10% $AlCl_3$ was added and incubated for 6 min in the dark. Sodium hydroxide (1 M, 1.0 mL) and distilled water (1.0 mL) were added to the mixture. The absorbance of each sample was measured at 510 nm using a UV-vis spectrophotometer (Agilent Technologies, USA). TFC was calculated by using a standard curve of Quercetin. TFC was expressed as mg Quercetin Equivalents (QE) per gram of dry plant material.

2.2.6. Determination of Sun Protection Factor (SPF)

All fractions were dissolved in methanol to prepare a solution of 2.0 mg/mL. Reference standard (A commercial sunscreen product) was also dissolved in methanol to obtain a 2.0 mg/mL solution. Methanol was used as the blank solution. The absorbance of each sample was measured from 290 nm–320 nm, at 5 nm intervals by using a microplate reader (ThermoFisher Scientific, USA 1510-01360C). *In vitro* Sun protection factor of each sample was determined by using Eq. (3),

$$SPF = CF \times \sum_{290}^{320} (\lambda)\, EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (3)$$

where *CF* is the correction factor equal to 10; *EE* (λ)is the Erythemal effect at wavelength λ; *I* (λ) is the solar intensity at wavelength λ; *Abs* (λ) is the absorbance of sunscreen product.[21]

The terms "*EE* (λ) × *I* (λ)" in the equation are constants. They were determined by *Sayre et al.* and are listed in Table 1.

Table 1. Correlation between the Erythemogenic Effect (EE) and solar intensity at the corresponding wavelength (I)

λ (nm)	EE (**λ**) × I (**λ**)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

2.2.7. Determination of antimicrobial activity

2.2.7.1. Preparation of pure cultures

The following cultures of bacteria and fungi were selected for the study. Gram-positive bacteria; *Staphylococcus aureus,* Gram-negative bacteria; *Klebsiella pneumoniae, Escherichia coli,* Fungi; *Candida albicans* were collected from the collection of the Department of Microbiology, University of Kelaniya. Fungus, *Aspergillus welwitschiae,* was obtained from the culture collection of the Department of Plant and Molecular Biology, University of Kelaniya. Bacterial cultures were inoculated on nutrient agar and incubated at 37 ^{0}C for 24 hours. The test fungal cultures were grown on potato dextrose agar medium and were incubated at room temperature (27 ^{0}C ± 2) for 3 days.

2.2.7.2. Evaluation of antibacterial activity by Agar disc diffusion method

Under sterile conditions, nutrient agar (25.0 mL) was poured into Petri dishes and allowed to solidify. Bacterial broth inoculum was adjusted by observing optical density at 600 nm, and 4.0 x 10^8 CFU/mL of inoculum was obtained. The bacterial suspension (100 µL) was spread throughout the plate and allowed to dry. A sterile filter paper disc (5 mm) was placed on agar plates and impregnated with the sample (1000 µg/mL), positive control – Amoxicillin (1000 µg/mL), and negative control - methanol (20 µL). Cultures were incubated at 37 ^{0}C for 24 h. Each sample was triplicated and measured inhibition zones to the nearest millimeter. All results were expressed in mm with Standard Deviation.

2.2.7.3. Evaluation of antifungal activity by disc diffusion method

Under sterile conditions, potato dextrose agar (25.0 mL) was poured into Petri dishes and allowed to solidify. Fungal spore inocula were adjusted to obtain 2.4 x 10^8 CFU/mL. Fungal spore suspension (100 µL) was spread throughout the plate and allowed to dry. Sterile filter paper discs (5 mm) were placed on agar plates and impregnated with the sample (1000 µg/mL, positive control – Carbendazim (250 µg/mL), and the negative control - methanol (20 µL). Cultures were incubated at 27 ^{0}C for 24 h. All samples were triplicated and measured inhibition zones to the nearest millimeter.[18]

2.2.8. Separation of compounds in DCM fraction using silica gel column chromatography

As the DCM fraction showed the highest antioxidant activity, UV protection, and antimicrobial activities with the highest amounts of TPC and TFC, it was selected for further separation and purification using silica gel column chromatography. The best solvent system was selected by thin-layer chromatography by testing different ratios of solvents (hexane, toluene, methanol, ethanol, ethyl acetate, acetone, glacial acetic acid, formic acid, distilled water, dichloromethane, etc.) Among the tested solvent systems, dichloromethane: ethyl acetate = 2: 3 was selected as the appropriate solvent system for TLC development.

A sample (0.10 g) of DCM fraction was dissolved in a minimum amount (10.0 mL) of DCM, and a column (internal diameter 1.2 cm) was packed with silica gel (10.0 g) by the slurry method. The column was cooled with DCM-moistened cotton to avoid cracking. The sample was subjected to column chromatography by gradient elution starting with DCM, followed by ethyl acetate, ethanol, and distilled water, and 50 fractions were collected. Those fractions were pooled into 6 sub-fractions (F1, F2, F3, F4, F5, and F6) by considering the similarity of R_f values in TLC profiles. Every TLC plate was observed under a UV lamp at 365 nm and 254 nm wavelengths. Each sub-fraction was dried and dissolved in methanol to obtain a 1 mg/mL solution, and their photoprotective properties were evaluated.

2.2.9. GC/MS analysis of the sub-fraction F3 separated by column chromatography

As the sub-fraction F3 was rich in chemical constituents with the highest photoprotective property, it was analyzed by gas chromatographic system (Agilent Technologies 7890B, USA) coupled with mass spectrometry (Agilent Technologies 5977B MSD, USA), capillary column (Agilent 44 19091s – 433 HP-5MS UI 5% phenyl methyl siloxane) having dimensions 30 m × 0.25 mm × 0.25 µm film thickness and a split injector. The initial column temperature was programmed as 40 ^{0}C.

Then the temperature was increased at a rate of 3 ^{0}C per minute to 120 ^{0}C and further increased to 260 ^{0}C at a rate of 5 ^{0}C per minute and held for 10 minutes. The injecting temperature was fixed at 260 ^{0}C. Helium was the carrier gas at a 3 mL/min flow rate and in splitless mode, injecting 5 µL of the sample. The volatile compounds in sub-fraction F3 were identified using the National Institute of Standards and Technology (NIST) library.[22]

2.2.10. Statistical analysis

All data were expressed as mean (± SD) for independent determinations in triplicate for each experiment. The results were analyzed statistically using GraphPad Prism 8.0.1 statistical software. Significant differences were assessed from Tukey's pairwise test after conducting ANOVA. Values were considered significant at $p < 0.05$. The correlation between the antioxidant activity of the fractions of methanolic extract of Kaffir lime leaves, and total phenolic and total flavonoid content was determined separately using Pearson's method. Correlations between the sun protection factor (SPF) in each fraction and the antioxidant activity, total phenolic content, and total flavonoid content were also determined by Pearson's method.

3. Results and Discussion

3.1. Extraction

The percentage yield of Greenish-yellow colored methanolic extract of Kaffir Lime leaves which was obtained using the maceration technique was 14.79%. Percentage yield may vary with the extraction method and solvent. *E. Husni* reported that the percentage yield of leaf essential oil was 0.91% by the hydrodistillation method, which was collected from Indonesia,[8] and macerated ethanolic extract from Thailand has been reported as 10.36%.[23]

Pumival *et al.* reported that the percent yield of chemical constituents extracted from Kaffir lime leaves from distillation was 0.71±0.08% v/w.[15] According to a study conducted at HCMC University of Technology in 2020, the percent yield of Kaffir lime leaf extract was 4.6% and hydro-distillation was used to extract the chemical constituents.[24]

Table 2. The respective weight and percentage yield of the methanol extract and each fraction

Sample	Weight of the sample (g) ± SD	Percentage yield (%) ± SD
Methanolic extract	4.39 ± 0.16	14.79 ± 0.55
Hexane fraction	0.09 ± 0.01	0.30 ± 0.02
DCM fraction	1.45 ± 0.11	4.89 ± 0.39
50% Methanol fraction	1.99 ± 0.16	6.72 ± 0.56

Among the fractions in this study, the 50% methanol fraction had the highest yield, followed by DCM and hexane. Results indicate that the Kaffir lime leaves contain more polar compounds and less amount of non-polar and moderately polar compounds.

3.2. Determination of antioxidant activity by DPPH free radical scavenging assay

Methanolic extract of Kaffir lime leaves (crude) and its three fractions exhibited DPPH free radical scavenging activity as shown in Fig. 1. In this assay, BHT, the positive control, showed the highest activity at 500 µg/mL. According to the results, among the fractions, DCM and methanol (50%) fractions showed the highest % inhibition at 2000 µg/mL. IC_{50} values of methanol extract of Kaffir lime leaves and its fractions are tabulated in Table 3.

Different letters (a–d) within the same column differ significantly ($p < 0.05$)

As shown in Table 3, the DCM fraction exhibited the highest radical scavenging activity among the fractions, with an IC_{50} of 186.20 ± 4.95 mg/mL. But it is less than the radical scavenging activity of BHT (IC_{50} = 15.84 ± 0.20 mg/mL). Methanolic extract (389.04 ± 6.29 mg/mL), 50% methanol (489.78 ± 1.51 mg/mL), and hexane (870.96 ± 2.08 mg/mL) fractions have less antioxidant properties. J. Wungsintaweekul *et al.* reported that the IC_{50} of *C. hystrix* leaf oil was >250 mg/mL and that of methanolic extract from the Soxhlet method was 24.6 mg/mL.[25] Another study from Malaysia stated that *C. hystrix* had the DPPH radical scavenging activity of 279.03 ± 10.34 µg/mL.[26]

As the phenolic compounds are mostly responsible for the antioxidant activity of natural products, the TPC of the subsequent fractions of methanolic extract of Kaffir lime leaves were determined and expressed in mg of Gallic acid equivalent per 1 g of dry weight as a mean of three replicates with SD and the results are tabulated in table 4.

Different letters (a–c) within the same column indicate significant difference at $p < 0.05$.

Table 3. The IC_{50} of methanol extract of Kaffir lime leaves and its fractions (each data point represents the mean of the 3 replicates as mean ± SD).

Sample	IC_{50} (µg/mL)
Methanolic extract	389.04 ± 6.29^{a}
Hexane fraction	870.96 ± 2.08^{b}
DCM fraction	186.20 ± 4.95^{c}
50% methanol fraction	489.78 ± 1.51^{d}
BHT	15.84 ± 0.20^{e}

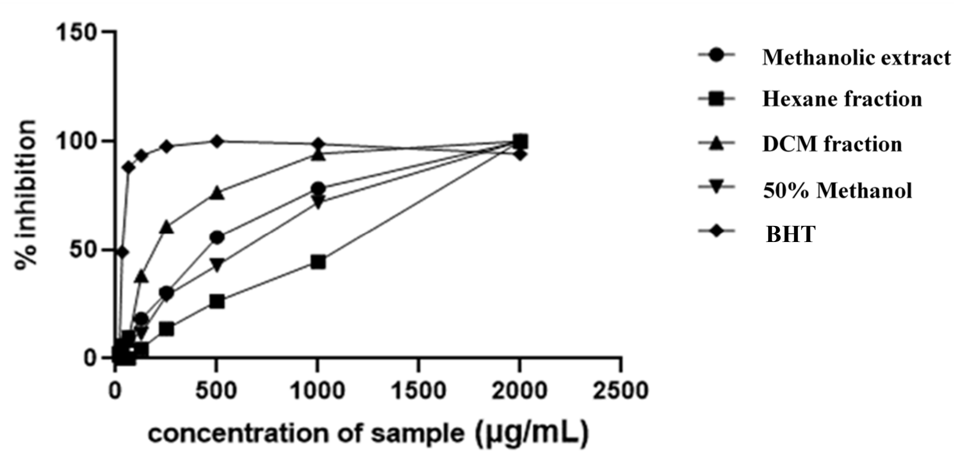

Fig. 1. Percent inhibition of methanolic extract (crude) of Kaffir lime leaves and its subsequent fractions

3.3. Total Phenolic Content

TPC of the samples was found to increase in the following order: 50% methanol fraction < hexane fraction < DCM fraction.

Among those fractions, the DCM fraction exhibited the highest TPC value of 0.64 ± 0.04 mg Gallic acid equivalents per g of dry weight of plant material. TPC of the Kaffir lime leaf extracts from Soxhlet extraction was reported by Ali *et al.* in the following order of methanol extract > ethanol extract > chloroform extracts. The TPC of methanol extract was 1.40 ± 0.32 mg/g.[27] The difference in the TPC order between the two studies may be attributed to the variation in extraction techniques.

Table 4. Total phenolic content of fractions of methanolic extract of Kaffir lime leaves

Fraction	TPC (mg/g)
Hexane fraction	0.53 ± 0.04^{a}
DCM fraction	0.64 ± 0.04^{b}
50% Methanol fraction	0.45 ± 0.03^{c}

3.4. Total Flavonoid Content

The total flavonoid content of fractions of methanolic extract of Kaffir lime leaves represented as mg of Quercetin per 1 g of dry weight are given in Table 5. All weights were tabulated as the mean of three replicates with standard deviation.

Table 5. Total flavonoid content of fractions of methanolic extract of Kaffir lime leaves

Fraction	TFC (mg/g)
Hexane fraction	3.72 ± 0.10^{a}
DCM fraction	5.03 ± 0.21^{b}
50% Methanol fraction	0.32 ± 0.01^{c}

Different letters (a–c) within the same column indicate significant differences at $p < 0.05$.

The total flavonoid contents of the samples were found to increase in the following order: 50% methanol fraction < hexane fraction < dichloromethane fraction. Among the fractions, the DCM fraction exhibited the highest TFC value of 5.03 ± 0.21 mg Quercetin equivalents per 1 g of dry weight of the plant material. TFC values of Kaffir lime leaves were previously reported to be decreased according to the order of methanol extract > chloroform extract > ethanol extracts when chemical constituents in leaves were Soxhlet extracted using different solvents.[27] According to another study, the highest TFC was recorded in the moderately polar ethyl acetate fraction as 0.1147 Rutin equivalents/mg. Flavonoids were decreased in the order of ethyl acetate > *n* –butanol > hexane.[28]

3.5. Sun Protection Factor (SPF) of methanolic extract of Kaffir lime leaves and its fractions

As the compounds with high photoprotective properties can be used for the formulation of sunscreens to avoid sunburns on the body, the SPF of the plant extract and its fractions were determined by a spectroscopic technique, and the corresponding SPF values are tabulated in Table 6.

Among the fractions of Kaffir lime leaf extract, the DCM fraction had the highest SPF value (34.04 ± 0.18) and which is greater than the reference sunscreen cream (33.49 ± 0.27). Sunscreen products can be categorized into three types based on their SPF values as a product that provides minimal (SPF <12), moderate (12 < SPF < 30), and high (SPF >30) sunburn protection.[29]

Table 6. SPF of methanolic extract of Kaffir lime leaves and its fractions

Sample (2 mg/mL)	SPF value
Methanolic extract	18.82 ± 0.18
Hexane fraction	5.96 ± 0.06
DCM fraction	34.03 ± 0.18
50% Methanol fraction	18.96 ± 0.21
Reference	33.48 ± 0.27

According to the results of this study, the DCM fraction had the highest UV protection ability and approximately blocks 97% of UV B rays. To the best of our knowledge, this is the first report on the photoprotective property of the leaf extract of kaffir lime.

3.6. Antimicrobial activity of methanolic extract of Kaffir lime leaves and its fractions by Disc diffusion method

The antimicrobial activity of Kaffir lime leaf extract and its fractions were determined by the disc diffusion method in triplicates. All fractions were dissolved in methanol; thus, methanol was used as the negative control. Amoxicillin was used as the positive control for bacterial strains, and Carbendazim was used as the positive control for antifungal studies. The inhibition zones (diameter) are expressed in mm with SD and tabulated in Table 7.

Methanolic extract of Kaffir lime leaves showed significant inhibition against all bacterial strains and fungal strain *Candida albicans* while small inhibition against *Aspergillus welwitschiae.*

The negative control showed no inhibition against all microbial strains. Among the fractions, the growth of *Escherichia coli* was inhibited by hexane and DCM fractions only. *Klebsiella pneumoniae*, *Staphylococcus aureus,* and *Candida albicans* were inhibited only by the DCM fraction. DCM fraction showed the highest inhibition against *Staphylococcus aureus* and *Klebsiella pneumonia*e with the zone of inhibition of 15.0 (±0.3) mm and 15.0 (±0.1) mm, respectively. *Aspergillus welwitschiae* was not inhibited by any fraction other than the methanolic extract.

Table 7. Zone of inhibition in diameter (mm) against tested microorganisms

	Methanolic extract	Hexane fraction	DCM fraction	50% Methanol fraction	Negative control	Positive control
Escherichia coli	10.0 (±0.5)	13.0 (±0.2)	10.0 (±0.2)	0.0	0.0	13.0 (±0.8)
Staphylococcus aureus	10.0 (±0.5)	0.0	15.0 (±0.3)	0.0	0.0	22.0 (±0.3)
Klebsiella pneumoniae	12.0 (±0.7)	0.0	15.0 (±0.1)	0.0	0.0	7.0 (±0.1)
Aspergillus welwitschiae	7.0 (±0.5)	0.0	0.0	0.0	0.0	17.0 (±0.1)
Candida albicans	8.0 (±0.7)	0.0	7.0 (±0.1)	0.0	0.0	8.0 (±0.1)

3.7. Correlation studies

Correlations of DPPH free radical scavenging activities (antioxidant activities) of fractions of methanol extract of lime leaves with TPC and TFC and correlation between SPF, and antioxidant activity, TPC, and TFC were determined by Pearson's method. Pearson's correlation is considered significantly negative when -0.61 < r < -0.9723 and significantly positive when 0.61 < r < 0.9715. Positive correlation is classified into five types according to their r values; very weak if the r value is 0.00-0.19, as weak if the r value is 0.2-0.39, as moderate if the r value is 0.40-0.59, as strong if the r value is 0.60-0.79 and as very strong if the r value is 0.8-1.0.[10] The correlation of the antioxidant activity with TPC and TFC is tabulated in Table 8.

Table 8. Correlation between antioxidant activity, TPC, and TFC

	$1/IC_{50}$ Vs. TPC	$1/IC_{50}$ Vs. TFC
Pearson's correlation coefficient (r)	0.7980	0.5593

A strong positive correlation indicated by a high r-value (r = 0.7980) was observed between TPC and DPPH antioxidant activity of the methanolic extract of Kaffir lime leaves. A positive moderate correlation was observed with a moderate r value (r = 0.5593) between TFC and DPPH antioxidant activity of methanolic extract of Kaffir lime leaves. The results suggested that phenolics in Kaffir lime leaves including flavonoids may contribute to the DPPH free radical scavenging activity of methanolic extract of leaves. The correlations between the SPF and DPPH free radical scavenging activity, TPC, and TFC are tabulated in Table 9.

A very strong positive correlation with a higher r-value (r = 0.9613) was observed between DPPH antioxidant activity and SPF value of fractions of Kaffir lime leaves. According to the results, it can be concluded that the compounds responsible for antioxidant properties may be responsible for the photoprotective property of the leaf extract. A strong positive correlation (r = 0.6011) was observed between SPF values and TPC values of extracts of Kaffir lime leaves. Therefore, it can be suggested that phenolic compounds may be responsible for the sun-protective property of Kaffir lime leaves. A weak positive correlation with low r-value (r = 0.3093) was observed between SPF values and TFC values of extracts of Kaffir lime leaves.

Table 9. Correlation between sun protection factor, antioxidant activity, TPC, and TFC

	SPF Vs. $1/IC_{50}$	SPF Vs. TPC	SPF Vs. TFC
Pearson's correlation coefficient (r)	0.9613	0.6011	0.3093

3.8. Separation of compounds in DCM fraction using silica gel column chromatography

Due to high SPF value, high antioxidant activity, and high antibacterial activity, the DCM fraction of methanolic extract was selected to be further separated by silica gel chromatography. Among the different solvent systems tested, the DCM: ethyl acetate 2:3 solvent system exhibited better separation, thus it was selected as the solvent system for column chromatography. After the separation, 50 fractions were collected and combined into 6 sub-fractions (F1, F2, F3, F4, F5, F6), considering their R_f values of TLC. The photoprotective properties of F1, F2, F3, F4, F5, and F6 sub-fractions were determined, and their corresponding SPF values are summarized in Table 10.

Table 10. SPF values of sub-fractions

Fractions after separated by column chromatography (DCM)	SPF value (1 mg/mL)
F1	26.55 ± 0.70
F2	25.88 ± 0.08
F3	28.10 ± 0.10
F4	25.27 ± 0.88
F5	16.72 ± 0.13
F6	14.50 ± 0.11

As the sub-fraction F3 exhibited the highest photoprotective property with the SPF value of 28.10 ± 0.10, it was further analyzed by GC-MS to identify its volatile constituents.

Sub-fraction F3 of the DCM fraction of Kaffir lime leaves contains at least 20 volatile compounds including linalool oxide, α-terpinolene, 2,4-bis(1,1-dimethylethyl)-phenol, diethyl phthalate, hexadecanoic acid, methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate and octadecanoic acid. According to a study conducted by a group at University Putra Malaysia, Kaffir lime leaf oil mainly contained β-Citronellal (6.59%) followed by linalool (3.90%) and citronellol (1.76%).[36] Peel oil contained mainly β-pinene, limonene, terpinene-4-ol, α-pinene, α-terpinene, γ-terpinene, and α-terpineol.[37] The reported bioactivities of the compounds identified by GC-MS are summarized in Table 12.

Table 11. Main volatile chemical constituents in the sub-fraction F3 with their respective retention time (minutes)

Retention time (mins)	Compound
14.6486	Linalool oxide [25]
19.9634	Alpha terpinolene [30]
32.6170	2,4-bis(1,1-dimethylethyl)-phenol [31]
34.2103	Diethyl phthalate [32]
42.3242	Hexadecanoic acid [33]
42.6795	Methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate [34]
47.3088	Octadecanoic acid [35]

Table 12. Chemical constituents with their reported bioactivities

Compound	Bioactivity
Linalool oxide	Antimicrobial, anti-inflammatory, anticancer, antioxidant properties, insect repellent, a lead compound in the synthesis of vitamins A and E,[38] absorbs UV at 243 nm and 272 nm[39]
Alpha terpinolene	Antiproliferative agent for brain tumor cells,[40] antifungal and antimicrobial properties, antioxidant properties for reducing LDL oxidation, anti-inflammatory and analgesic effects[41]
2,4-bis(1,1-dimethylethyl)-phenol	Antibacterial, antifungal and antiviral activities, insecticidal, herbicidal and nematocidal activity, antioxidant activity, anti-inflammatory activity, cytotoxicity[42] as a UV absorber and stabilizer[43]
Diethyl phthalate	Antimicrobial activity[44] allelopathic, antimicrobial, insecticidal activities[45]
Hexadecanoic acid	Antioxidants, hypocholesterolemic activity, nematicide, as a pesticide[46] anticancer activity and anti-inflammatory activity[47]
Methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	Antioxidant[48]
Octadecanoic acid	Anti-inflammatory, antibacterial, hypocholesterolemic, and hepatoprotective activities, intestinal lipid metabolism regulation, nematicidal, antinociceptive, antioxidant, and antifungal properties[47]

4. Conclusions

This study was conducted to explore the antioxidant, photoprotective, and antimicrobial activities of the chemical constituents in the methanolic extract of Kaffir lime leaves. After the maceration, the methanolic extract was fractionated by the solvent extraction method with hexane, DCM, and 50% methanol. Then, each fraction was tested to determine its TPC, TFC, DPPH free radical scavenging activity, photoprotective property, antibacterial activity, and antifungal activity against selected strains.

Among the fractions, The DCM fraction exhibited the highest TPC (0.64 ± 0.04 mg/g), TFC (5.03 ± 0.21 mg/g), antioxidant activity (186.20 ± 4.95 µg/mL), photoprotective property (SPF- 34.03 ± 0.18) and antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Candida albicans*. Thus, the DCM fraction was further separated into 6 sub-fractions by column chromatography, and the photo-protective property of each subfraction was determined. As subfraction F3 exhibited the highest SPF value (28.10 ± 0.10) it was subjected to GC-MS analysis to identify its volatile constituents and it was found to be rich in linalool oxide, α-terpinolene, 2,4-bis(1,1-dimethylethyl)-phenol, diethyl phthalate, hexadecanoic acid, methyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate and octadecanoic acid.

As the results revealed that the DCM fraction of the Kaffir lime leaves extract was rich in phytochemicals with antioxidant, antimicrobial and high photoprotective properties, DCM can be used as a solvent to extract the compounds responsible for the bioactivities and further research should be carried out to isolate and characterize its bioactive compounds. As for future work, each of the active fraction/s will be separated and purified by HPLC and column chromatography, and the structures of the isolated compounds that are responsible for the antioxidant, antimicrobial, and photoprotective properties can be determined by NMR, FT-IR, HPLC, UV-vis spectroscopy, and GC-MS techniques. The potential use of chemical constituents with photoprotective properties, antimicrobial properties, and antioxidant properties in the pharmaceutical and cosmetics industry will be further examined.

Acknowledgements

This work was supported by the University of Kelaniya, Sri Lanka, under the research grant number RP/03/02/06/01/2018. The authors would like to acknowledge Amila T. Kannangara for supporting the GC/MS analysis of samples.

References

[1] Budiarto, R.; Sholikin, M. M. Kaffir Lime Essential Oil Variation in the Last Fifty Years: A Meta-Analysis of Plant Origins, Plant Parts and Extraction Methods. *Horticulturae* **2022**, *8*, 1132. https://doi.org/10.3390/horticulturae8121132

[2] Suresh, A.; Velusamy, S.; Ayyasamy, S.; Rathinasamy, M. Techniques for Essential Oil Extraction from Kaffir Lime and its Application in Health Care Products—A Review. *Flavour Fragr J.* **2021**, *36*, 5–21. https://doi.org/10.1002/ffj.3626

[3] Ali, M.; Akhter, R.; Narjish, S. N.; Shahriar, M.; Bhuiyan, M. A. Studies of Preliminary Phytochemical Screening, Membrane Stabilizing Activity, Thrombolytic Activity and *in-vitro* antioxidant activity of leaf extract of Citrus Hystrix. *Int. J. Pharm. Sci. Res.* **2015**, *6*, 2367–2374. http://doi.org/10.13040/IJPSR.0975-8232.6(6).2367-74

[4] Butryee, C.; Sungpuag, P.; Chitchumroonchokchai, C. Effect of Processing on the Flavonoid Content and Antioxidant Capacity of *Citrus hystrix* Leaf. *Int J Food Sci Nutr*. **2009**, *60*, 162–174. https://doi.org/10.1080/09637480903018816

[5] Husni, E.; Putri, U. S.; Dachriyanus. Chemical Content Profile of Essential Oil from Kaffir Lime (*Citrus hystrix* DC.) in Tanah Datar Regency and Antibacterial Activity. In *Proc 2nd Int. Conf. Contemp. Sci. Clin. Pharm. 2021 (ICCSCP 2021)*; pp 174–181. https://doi.org/10.2991/ahsr.k.211105.025

[6] Budiarto, R.; Poerwanto, R.; Santosa, E.; Efendi, D.; Agusta, A. Preliminary Study on Antioxidant and Antibacterial Activity of Kaffir Lime (*Citrus hystrix* DC) Leaf Essential Oil. *Applied Research in Science and Technology* **2021**, *1*, 58–65. https://doi.org/10.33292/areste.v1i2.8

[7] Abirami, A.; Nagarani, G.; Siddhuraju, P. Antimicrobial Activity of Crude Extract of *Citrus Hystrix* and *Citrus Maxima*. *Int. J. Pharm. Sci. Res*. **2013**, *4*, 1–5. http://dx.doi.org/10.13040/IJPSR.0975-8232.4(1).296-00

[8] Masruri, M.; Azhar, A. Z.; Rosyada, I.; Febrianto, A. The Effect of Kaffir Lime *(Citrus hystrix* DC) Essential Oil on Bioplastic Derived from Cassava Peel Waste. *J. Phys. Conf. Ser.* **2019**, *1374*, 012015. http://doi.org/10.1088/1742-6596/1374/1/012015

[9] Budiarto, R.; Wathoni, N.; Mubarok, S.; Hamdani, J. S.; Ujilestari, T.; Darsih, C.; Sari, R. M.; Rukmana, R. M.; Sholikin, M. M. Recent Overview of Kaffir Lime (*Citrus hystrix* DC) Dual Functionality in Food and Nutraceuticals. *J. Agric. Res.* **2024**, *18*, 101384. https://doi.org/10.1016/j.jafr.2024.101384

[10] Anuchapreeda, S.; Chueahongthong, F.; Viriyaadhammaa, N.; Panyajai, P.; Anzawa, R.; Tima, S.; Ampasavate, C.; Saiai, A.; Rungrojsakul, M.; Usuki, T.; *et al.* Antileukemic Cell Proliferation of Active Compounds from Kaffir Lime (*Citrus hystrix*) Leaves. *Molecules* **2020**, *25*, 1300. https://doi.org/10.3390/molecules25061300

[11] Hien, T. T.; Quyen, N. T. C.; Truc, T. T.; Quan, P. M. Evaluate the Chemical Composition of Kaffir Lime (*Citrus hystrix*) Essential Oil Using the Classical Method. *IOP Conf. Ser.: Mater. Sci. Eng.* **2020**, *991*, 012014. https://doi.org/10.1088/1757-899X/991/1/012014

[12] Harshani, H. S.; Karunaratne, M. M. S. C. Volatile Profiling and Bio-Efficacy of *Citrus hystrix* Fruit Peel as a Seed Protectant against *Callosobruchus maculatus*. *J. Entomol. Zool. Stud.* **2018**, *6*, 27–31.

[13] Budiarti, L. Y.; Wydiamala, E.; Madani, R. A. Antibacterial Activity of Extract Combination of Leaves and Peels Kaffir Lime (*Citrus Hystrix* DC.) Against Some Test Bacteria. *Bioinformatics and Biomedical Research Journal* **2021**, *4*, 39–47. https://doi.org/10.11594/bbrj.04.02.01

[14] Uda, M. N. A.; Harzana Shaari, N.; Shamierasaid, N.; Ibrahim, N. H.; Akhir, M. A. M.; Hashim, M. K. R.; Salimi, M. N.; Nuradibah, M. A.; Hashim, U. *et al.* Antimicrobial Activity of Plant Extracts from Aloe Vera, Citrus Hystrix, Sabah Snake Grass and Zingiber Officinale against Pyricularia Oryzae that causes Rice Blast Disease in Paddy Plants. *IOP Conf. Ser. Mater. Sci. Eng*. **2018**, *318*, 012009. htpps://doi.org/10.1088/1757-899X/318/1/012009

[15] Pumival, P.; Tadtong, S.; Athikomkulchai, S.; Chittasupho, C. Antifungal Activity and the Chemical and Physical Stability of Microemulsions Containing *Citrus hystrix* DC Leaf Oil. *Nat. Prod. Commun*. **2020**, *15*, 1–12. https://doi.org/10.1177/1934578X20957755

[16] Aziman, N.; Abdullah, N.; Mohd Noor, Z.; Zulkifli, K. S.; Wan Kamarudin, W. S. S. Phytochemical Constituents and *in vitro* Bioactivity of Ethanolic Aromatic Herb Extracts. *Sains. Malaysiana* **2012**, *41*, 1437–1444.

[17] Chatatikun, M.; Chiabchalard, A. Phytochemical Screening and Free Radical Scavenging Activities of Orange Baby Carrot and Carrot (Daucus carota Linn.) Root Crude Extracts. *J. Chem. Pharm. Res*. **2013**, *5*, 97–102.

[18] Dewage, D. D. D. H.; Karunaratne, S. H. S.; Rajapakse, C. S. K. Guava (*Psidium guajava* L.) Leaves as a Source of Phytochemicals with Antioxidant, Antimicrobial and Photoprotective Properties for Sunscreen Formulations. *Asian J. Chem*. **2022**, *35*, 89–94. https://doi.org/10.14233/ajchem.2023.24058
[19] Asma, F.; Eddine, H. S.; Yassmine, C.; Hanane, Z. Phytochemical Screening, Antibacterial and Antioxidant Activities of *Ocimum Basilicum* L. Cultivated in Biskra, Algeria. *Chem. Chem. Technol.* **2023**, *17*, 397–406. https://doi.org/10.23939/chcht17.02.397
[20] Uduwela, U. D. H. K.; Deraniyagala, S. A.; Thiripuranathar, G. Antioxidant and Anti-Inflammatory Potential of the Aqueous Extract of the Peel of a Sri Lankan Variety of *Nephelium lappaceum* Linn. *World J. Pharm. Res*. **2019**, *8*,154–166. DOI: 10.20959/wjpr20192-14177
[21] Zarkogianni, M.; Nikolaidis, N. Determination of Sun Protection Factor (SPF) and Stability of Oil-in-Water Emulsions Containing Greek Red Saffron (Crocus Sativus L.) as a Main Antisolar Agent. *Int. J. Adv. Res. Chem. Sci*. **2016**, *3*, 1–7. http://dx.doi.org/10.20431/2349-0403.0307001
[22] Zheng, L.-Y.; Sun, G.-M.; Liu, Y.-G.; Lv, L.-L.; Yang, W.-X.; Zhao, W.-F.; Wei, C.-B. Aroma Volatile Compounds from Two Fresh Pineapple Varieties in China. *Int. J. Mol. Sci.* **2012**, *13*, 7383–7392. https://doi.org/10.3390/ijms13067383
[23] Chueahongthong, F.; Ampasavate, C.; Okonogi, S.; Tima, S. Cytotoxic Effects of Crude Kaffir Lime (*Citrus hystrix*, DC.) Leaf Fractional Extracts on Leukemic Cell Lines. *J. Med. Plants Res*. **2011**, *5*, 3097–3105.
[24] Le, X. T.; Ha, P. T. H.; Phong, H. X.; Hien, T. T.; Ngan, T. T. K. Extraction of Essential Oils and Volatile Compounds of Kaffir Lime (*Citrus hystrix* D.C) by Hydrodistillation Method. *IOP Conf. Ser. Mater. Sci. Eng*. **2020**, *991*, 012024. https://doi.org/10.1088/1757-899X/991/1/012024
[25] Wungsintaweekul, J.; Sitthithaworn, W.; Putalun, W.; Pfeifhoffer, H. W.; Brantner, A. Antimicrobial, Antioxidant Activities and Chemical Composition of Selected Thai Spices. *Songklanakarin J. Sci. Technol*. **2010**, *32*, 589–598.
[26] Othman, H. I. A.; Alkatib, H. H.; Zaid, A.; Sasidharan, S.; Rahiman, S. S. F.; Lee, T. P.; Dimitrovski, G.; Althakafy, J. T.; Wong, Y. F. Phytochemical Composition, Antioxidant and Antiproliferative Activities of *Citrus hystrix, Citrus limon, Citrus pyriformis,* and *Citrus microcarpa* Leaf Essential Oils against Human Cervical Cancer Cell Line. *Plants* (Basel) **2022**, *12*, 134. https://doi.org/10.3390/plants12010134
[27] Ali, M.; Akhter, R.; Narjish, S. N.; Shahriar, M.; Bhuiyan, M. A. Studies of Preliminary Phytochemical Screening, Membrane Stabilizing Activity, Thrombolytic Activity and *in-vitro* Antioxidant Activity of Leaf Extract of *Citrus hystrix*. *Int. J. Pharm. Sci. Res.* **2015**, *6*, 2367–2374. http://doi.org/10.13040/IJPSR.0975-8232.6(6).2367-74
[28] Wijaya, Y. A.; Widyadinata, D.; Irawaty, W.; Ayucitra, A. Fractionation of Phenolic Compounds from Kaffir Lime (Citrus hystrix) Peel Extract and Evaluation of Antioxidant Activity. *Reaktor* **2017**, *17*, 111–117. https://doi.org/10.14710/reaktor.17.3.111-117
[29] Abirami, A.; Nagarani, G.; Siddhuraju, P. The Medicinal and Nutritional Role of Underutilized Citrus Fruit-*Citrus hystrix* (Kaffir lime): A Review. *Drug Invent. Today* **2014**, *6*, 1–5.
[30] Webbook.nist.gov. Cyclohexene, 1-methyl-4-(1-methylethylidene)-. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=586-62-9 (accessed 2024-09-02).
[31] Webbook.nist.gov. 2,4-Di-tert-butylphenol. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=C96764&Mask=8 (accessed 2024-09-02).
[32] Webbook.nist.gov. Diethyl Phthalate. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=84-66-2 (accessed 2024-09-02).
[33] Webbook.nist.gov. Hexadecanoic acid, methyl ester. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=112-39-0 (accessed 2024-09-02).
[34] Webbook.nist.gov. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=6386-38-5 (accessed 2024-09-02).
[35] Webbook.nist.gov. Methyl stearate. Published online 2022. https://webbook.nist.gov/cgi/cbook.cgi?ID=C112618&Mask=8 (accessed 2024-09-02).
[36] Loh, F. S.; Awang, R. M.; Omar, D.; Rahmani, M. Insecticidal Properties of Citrus hystrix DC Leaves Essential Oil against Spodoptera litura fabricius. *J. Med. Plants Res*. **2011**, *5*, 3739–3744.
[37] Borusiewicz, M.; Trojanowska, D.; Paluchowska, P.; Janeczko, Z.; Petitjean, M. W.; Budak, A. Cytostatic, Cytotoxic, and Antibacterial Activities of Essential Oil Isolated from *Citrus hystrix*. *ScienceAsia* **2017**, *43*, 96–106
[38] Agrawal, P. K.; Berlinck, R. G. D. S. *Natural Product Communications*, 2014.
[39] Weast, R. C. (ed.). *Handbook of Chemistry and Physics. 60th Ed*; CRC Press Inc.: Boca Raton, Florida, 1979.
[40] Aydin, E.; Türkez, H.; Taşdemir, S. Anticancer and Antioxidant Properties of Terpinolene in Rat Brain Cells. *Archives of Industrial Hygiene and Toxicology* **2013**, *64*, 415–424. https://doi.org/10.2478/10004-1254-64-2013-2365
[41] Abstrax Tech. What is Terpinolene and What Are Its Benefits? 2022. https://abstraxtech.com/blogs/learn/what-is-terpinolene-and-what-are-its-benefits#:~:text=Terpinolene%20Has%20Antimicrobial%20and%20Antifungal,success%20is%20due%20to%20terpinolene. (accessed 2024-09-02).
[42] Ho, Y.; Suphrom, N.; Daowtak, K.; Potup, P.; Thongsri, Y.; Usuwanthim, K. Anticancer Effect of *Citrus hystrix* DC. Leaf Extract and Its Bioactive Constituents Citronellol and, Citronellal on the Triple Negative Breast Cancer MDA-MB-231 Cell Line. *Pharmaceuticals* **2020**, *13*, 476. https://doi.org/10.3390/ph13120476
[43] Information NC for B. Compound Summary for CID 7311, 2,4-Di-tert-butylphenol. *PubChem*. Published online 2022. https://pubchem.ncbi.nlm.nih.gov/compound/2_4-Di-tert-butylphenol

[44] Premjanu, N.; Jaynthy, C. Antimicrobial Activity of Diethyl Phthalate: An Insilico Approach. *Asian J. Pharm. Clin. Res.* **2014**, *7*, 141–142.

[45] Huang, L.; Zhu, X.; Zhou, S.; Cheng, Z.; Shi, K.; Zhang, C.; Shao, H. Phthalic Acid Esters: Natural Sources and Biological Activities. *Toxins* **2021**, *13*, 495. https://doi.org/10.3390/toxins13070495

[46] Siswadi, S.; Saragih, G. S. Phytochemical Analysis of Bioactive Compounds in Ethanolic Extract of Sterculia quadrifida R.Br. *AIP Conf. Proc.* **2021**, *2353*, 030098. https://doi.org/10.1063/5.0053057

[47] Mazumder, K.; Nabila, A.; Aktar, A.; Farahnaky, A. Bioactive Variability and *in vitro* and *in vivo* Antioxidant Activity of Unprocessed and Processed Flour of Nine Cultivars of Australian lupin Species: A Comprehensive Substantiation. *Antioxidants* **2020**, *9*, 282. https://doi.org/10.3390/antiox9040282

[48] Li, X.; Wang, Z. G.; Chen, H. H.; Liu, S. G. The Antioxidant Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate. *Acta Crystallogr. C. Struct. Chem.* **2014**, *70*, 1050-1053. https://doi.org/10.1107/S2053229614021445

Received: September 22, 2024 / Revised: January 24, 2025 / Accepted: February 07, 2025

РОЗКРИТТЯ БІОЛОГІЧНО АКТИВНОГО ПОТЕНЦІАЛУ ЛИСТЯ КАФРСЬКОГО ЛАЙМА (*CITRUS HYSTRIX*): АНТИОКСИДАНТНІ, АНТИМІКРОБНІ ТА ФОТОЗАХИСНІ ВЛАСТИВОСТІ

Анотація. *Кафрський лайм* (*Citrus hystrix*), *вид цитрусових, що походить з Південно-Східної Азії, має обмежені дослідження щодо свого фармацевтичного та космецевтичного потенціалу. Це дослідження вивчає антиоксидантні, антимікробні та фотозахисні властивості метанольного екстракту листя кафрського лайма. Результати показують, що листя кафрського лайма є перспективним рослинним джерелом для фармакологічного застосування, оскільки воно багате на фітохімічні речовини з антиоксидантним потенціалом, сильними фотозахисними властивостями з високою величиною SPF та антимікробною активністю щодо* *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Candida albicans* *та* *Aspergillus welwitschiae.*

Ключові слова: *антиоксидантні, протимікробні, фармакологічні, фотозахисні.*