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**Pharmaceutical and cosmeceutical potential of methanolic extract of Kaffir lime  
(*Citrus hystrix*) leaves**

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Kaffir lime (*Citrus hystrix*), commonly known as 'gada dehi' is a citrus fruit native to Southeast Asia. As less research has been conducted to investigate the potential pharmaceutical and cosmeceutical properties, the present study aimed to evaluate the antioxidant, antimicrobial and photo-protective properties of methanolic extract of Kaffir lime leaves. Dried and powdered Kaffir lime leaves were extracted into methanol by maceration. Constituents in the extract were then sequentially fractionated with hexane, dichloromethane (DCM), and methanol (50%). The antioxidant activity of each fraction was evaluated by  $\alpha$ -diphenyl-  $\beta$ - picrylhydrazyl (DPPH) free radical scavenging assay. Total phenolic content (TPC) and total flavonoid content (TFC) of each fraction were determined by Folin-Ciocalteu and aluminium chloride colorimetric assays respectively. The antibacterial and antifungal activities of methanolic extract of leaves and its fractions were determined against selected strains of bacteria *Klebsiella pneumonia*, *E.coli*, *Staphylococcus aureus*, and fungi *Aspergillus welwitschiae*, *Candida albicans* by agar disc diffusion method. The photoprotective property of the methanolic extract and its fractions were assessed spectrophotometrically by determining the Sun Protection Factor (SPF) using the Mansur equation. Among the fractions, the DCM fraction showed the highest antioxidant activity ( $IC_{50}$   $186.20 \pm 4.95$   $\mu$ g/ml) with the highest TPC and TFC ( $0.64 \pm 0.04$  mg gallic acid equivalent/g of dried weight and  $5.03 \pm 0.21$  mg of quercetin equivalent/g of dried weight, respectively). The methanolic extract showed inhibition against all the tested bacteria and fungi and among the fractions, the DCM fraction exhibited the highest inhibition against *K. pneumonia*, *S. aureus*, and *C. albicans*. All the fractions exhibited UV-B absorption, and among them, DCM showed the highest photo-protective property with an SPF of  $34.03 \pm 0.18$  at 2 mg/ml while the SPF of the reference sunscreen was  $33.48 \pm 0.27$  at 2 mg/ml. As the DCM fraction exhibited the highest antioxidant and photoprotective properties and significant antimicrobial activity, it was further separated by silica gel column chromatography into 6 subfractions. Further, the subfraction 3 (F3) was analysed by GC-MS as it contained the chemical constituents with the highest photoprotective property among the 6 subfractions and revealed that it is rich in volatile constituents including linalool oxide,  $\alpha$ -terpinolene, 2,4-bis(1,1-dimethylethyl)-phenol, diethyl phthalate, hexadecanoic acid, methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl) propionate and octadecanoic acid. These findings demonstrated that the extract of Kaffir lime leaves could be used as a natural source in the pharmaceutical and cosmeceutical industries as it is rich in phytochemicals with antioxidant, antimicrobial activities, and photoprotective properties with a high sun protection factor.

**Keywords** – Antioxidant, Antimicrobial, Photoprotective, Phytochemicals, Sunscreen

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