Jurnal Pharmascience, Vol. 12, Issue 2, October 2025, page: 493-501
ISSN-Print 2355 – 5386
ISSN-Online 2460-9560
https://dx.doi.org/10.20527/jps.v12i2.23081
Research Article

GC-MS Profiling and Inhibitory Effect on UV-C Exposure Induced Eryrhrocyte Damage of Sungkai Leaf: A Potential Novel Antioxidant Agent

Mashuri[1], Eko Suhartono[2,3]*, Noor Muthmainah[4], Lisda Hayatie[4], Nadia Salma Nazwa Assyfa[5], Hanna Dwi Aprilia Husna[5], Helma Febrianti[5]

[1]Department of Radiology, Faculty of Medicine and Health Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia
[2] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Health Science, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia
[3]Health Research Center for Wetland Faculty of Medicine and Health Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia
[4]Department of Microbiology anda Parasitology, Faculty of Medicine and Health Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia
[5]Medical Graduate, Faculty of Medicine and Health Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia
Email: esuhartono@ulm.ac.id

ABSTRAK

Daun sungkai (Peronema canescens Jack.) meruapakan tumbuhan yang dikenal memiliki berbagai manfaat farmakologis, termasuk sebagai antiinflamasi, antimikroba, dan antioksidan. Tumbuhan ini mengandung senyawa fenolik, flavonoid, tanin, dan saponin yang dapat berperan seabagai antioksidan. Berkaitan dengan hal tersebut, penelitian ini bertujuan untuk menganalisis profil fitokimia dengan GC-MS. Selain itu, juga akan membuktikan aktivitas antioksidan ekstrak daun sungkai dalam menhambat kerusakan membran eritorit akibat pajanan sinar UV. Daun Sungkai diperoleh dari KHDTK Mandiangin diektrak dengan pelarut methanol dengan metode maserasi. Hasil ektrak dilakukan uji GC-MS dan uji penghambatan kerusakan eritrosit akibat sinar UV. Pengujian terdiri atas 2 kelompok, yani 2 kelompok control dan 3 kelompok perlakuan. Kelompok perlakuan menggunakan ektrak metanol daun sungkai masing-masing 10 ppm, 15 ppm, dan 25 ppm. Sementara itu, pemajanan eritrosit dilakukan selama 2 jam. Hasil penelitian ini dapat disimpulkan bahwa berdasarkan analisis GC MS menunjukkan bahwa ekstrak metanol daun sungkai mengandung senyawa 4-Metoksi-3-trimetilsilil)metil-1-oktena, D-Limonen, Asam tridekanoat, 12-cis-oktadekanoat, asam 13-oktadesenoat, metil ester, asam oleat, dan Glisidil (Z) -9-heptadekanoat yang dapat mengurangi kerusakan eritrosit akibat paparan sinar UV. Selain itu, ektrak metanol daun sungkai dapat menghambat kerusakan membrane eritrosit yang ditandai oleh penurunan %Hb dan kadar MDA pada kelompok yang diberikan ekstrak methanol daun insulin.

Kata Kunci: Pajaanan UV, antioksidan, Daun Sungkai, Membrane Eritrosit, Peroksidasi Lipid

ABSTRACT

Sungkai leaf (*Peronema canescens* Jack.) is a plant known to have various pharmacological benefits, including as anti-inflammatory, antimicrobial, and antioxidant. This plant contains phenolic compounds, flavonoids, tannins, and saponins that can act as antioxidants. In this regard, this study aims to analyze the phytochemical profile by GC-MS. In addition, it will also prove the antioxidant activity of sungkai leaf extract in inhibiting erythrocyte membrane damage due to UV exposure. Sungkai leaves obtained from KHDTK Mandiangin were extracted with methanol solvent by maceration method. The extracts were subjected to GC-MS test and UV light-induced erythrocyte damage inhibition test. The test consisted of 2 groups, 2 control groups and 3 treatment groups. The treatment groups used methanol extract of sungkai leaves at 10 ppm, 15 ppm, and 25 ppm, respectively. Meanwhile, erythrocyte exposure was carried out for 2 hours. The results of this study can be concluded that based on GC MS analysis shows that the methanol extract of sungkai leaves contains compounds 4-Methoxy-3-trimethylsilyl)methyl-1-octene, D-Limonen, Tridecanoic acid, 12-cis-octadecanoic, 13-octadesenoic acid, methyl ester, oleic acid, and Glycidyl (Z)-9-heptadecanoic which can reduce erythrocyte damage due to UV exposure. In addition, sungkai leaf methanol extract can inhibit erythrocyte membrane damage characterized by a decrease in %Hb and MDA levels in the group given insulin leaf methanol extract.

Keywords: UV Exposure, Antioxidant, Sungkai Leaf, Erythrocyte Membrane, Lipid Peroxidation

I. INTRODUCTION

South Kalimantan is a tropical region of Indonesia that is geographically located near the equator, thus receiving a relatively high intensity of ultraviolet (UV) radiation throughout the year (Gemayel, 2013). This geographical position causes South Kalimantan to have a UV index at high to very high levels. This condition can potentially increase the risk of skin damage, premature aging, and skin cancer (Kruttman, et al., 2019; Mousavi, et al., 2024).

UV light consists of three types, namely UVC (λ= 100-280 nm), UVB (λ= 280-320 nm) and UVA (λ=320-400 nm). UVC has the lowest wavelength, so it has the most energy, so it can change the electronic structure of triplet oxygen ($^3O_2$) to singlet ($^1O_2$). The singlet oxygen is a non-radical reactive oxygen (ROS). Research by Purnamasari and Widiyanto (2023) states that UV exposure can increase the number of skin netrophils through its reaction with ROS. Another study also mentioned that UV exposure can cause

hemolysis of rat erythrocytes (Morazzoni et al., 2000).

Hemolysis in UV-induced erythrocytes starts from the oxidation of the erythrocyte lipid membrane by $^1O_2$ (Sokolova et al., 2023). The oxidation of the lipid membrane causes lipid peroxidation, so that membrane fluidity is disrupted. This results in impaired transmembrane fluid transport, resulting in swelling of the erythrocyte volume until lysis occurs. (Maltar-Strmečki et al., 2020; Kökbaş et al., 2021)

Oxidative damage to the erythrocyte membrane due to ROS from UV exposure can be inhibited by natural antioxidants, namely sungkai leaves (Peronema canescens Jack.). This plant is known to have various pharmacological benefits, including as anti-inflammatory, antimicrobial, and antioxidant (Latifah et al., 2024; Okfrianti et al., 2020). Various studies have shown that sungkai leaf extract contains active compounds such as flavonoids, tannins, saponins, and phenolics that act as antioxidants (Emilia et al., 2023). These compounds simultaneously work by inhibiting ROS so that erythrocyte damage can be prevented. In addition to these compounds, there are still many other compounds that are not yet known, especially the GC MS profile of sungkai leaves. In addition, the study of the mechanism of inhibition of erythrocyte damage due to UV exposure by sungkai leaves is still not widely known. Thus, the research was conducted to determine the phytochemical profile of GC MS and analyze the role of sungkai leaves in inhibiting erythrocyte damage.

II. METHOD

A. Erythrocyte Preparation

Erythrocytes were obtained from volunteers at PMI Banjar Regency with the approval of the Research Ethics Committee with No. 210/KEPK-FKIK ULM/EC/X/2024 dated October 24, 2024. Erythrocytes obtained were then washed with 6.8% phosphate buffer with pH 7.4.

B. Plant Identification

Sungkai leaves obtained from Mandiangin Forest KHDTK have been identified at the Basic Laboratory of Mathematics and Natural Sciences ULM with No. 201c/LB.LABDASAR/IX/2024 dated September 9, 2024. The identification results stated that the plant is *Peronema conescens* Jack.

C. Extraction of Sungkai Leaves

Extraction of Sungkai leaves used maceration method with 70% methanol solvent as done by Yusanto et al (2023) A total of 2 kg of dry weight of Sungkai leaves obtained 0.8 grams of Sungkai methanol extract (MES). After that, 0.1 gram of

extract was taken to test the GC-MS profile of Sungkai methanol extract at the ULM Integrated Laboratory.

D. Ultraviolet Exposure on Rrythrocytes

Erythrocytes were divided into five research groups consisting of two control groups and three treatment groups with five test tubes each as repetition. The treatment division is as follows. P0(-): Negative control group, 0.5 mL erythrocytes added 0.5 mL NaCl 0.9% without UV exposure; P0(+): 0.5 mL erythrocytes to which 0.5 mL of 0.9% NaCl was added; P1: 0.5 mL erythrocytes to which 0.5 mL of methanol extract of sungkai leaves with concentration of 10 ppm in 1% CMC solution; P2: 0.5 mL erythrocytes to which 0.5 mL of methanol extract of sungkai leaves with concentration of 15 ppm in 1% CMC solution; P3: 0.5 mL erythrocytes to which 0.5 mL of methanol extract of sungkai leaves with concentration of 25 ppm in 1% CMC solution. All groups were incubated for 30 minutes, after which they were put in a reactor to be irradiated for 2 hours.

E. Measurement of Erythrocyte Membrane Damage

Erythrocyte membrane damage is measured by determining Hb levels, as done by Amrullah et al (2016). Hb measurement using the Sianmethemoglobin method.

F. Measurement of Lipid Peroxidation

Lipid peroxidation is used as an indicator of increased levels of stable malondialdehyde (MDA), MDA measurement using the TBARS method with spectrophotometry at a wavelength of λ = 532 nm. MDA measurements were carried out by methods such as previous research (Suhartono et al., 2015).

G. Data Analysis

The data obtained were tested for normality with the help of the web-based statistical application https://www.gigacalculator.com/calculators/normality-test-calculator.php . Meanwhile, further tests used the Kruskal Wallis test which was calculated with the help of the web-based statistical application https://www.socscistatistics.com/tests/kruskal/default.aspx.

III. RESULTS AND DISCUSSION

The phytochemical profile by GC MS can be seen in Figure 1.

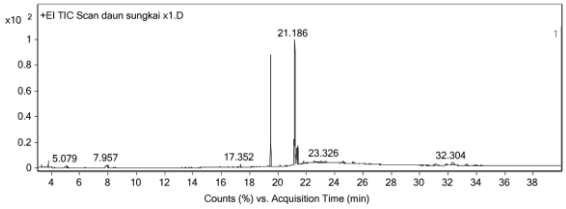

Figure 1. GC MS profile of methanol extract of Sungkai leaves

Based on Figure 1, the phytochemical content profile of sungkai leaf methanol extract can be seen as in Table I.

The RT value indicates the retention time, which is the time taken by the compound to exit the chromatographic column and be detected by the detector; RT is specific to each compound depending on its volatility and interaction with the stationary phase of the column. Meanwhile, % Area represents the percentage area of a compound's chromatogram peak compared to the total area of all peaks detected in a single analysis. This parameter is used to estimate the relative content of compounds in natural samples.

Phytochemical compounds of Sungkai leaf methanol extract can prevent erythrocyte membrane damage as in the following research results (Table II).

Table I. Phytochemical content profile of sungkai leaf methanol extract

RT	Area (%)	Formula	Name
5.079	2.31	C13H28OSi	4-Methoxy-3-(trimethylsilyl)methyl-1-octene
7.957	2.88	C10H16	D-Limonen
17.352	0.56	C15H30O2	Tridecanoic acid, 12-cis-octadecadienoate
21.186	35.26	C19H36O2	13-Octadecanoic acid, methyl ester
23.326	0.81	C18H35O2	Oleic acid
32.304	10.06	C20H36O3	Glycidyl(Z)-9-heptadecanoate

Table II. Erythrocyte membrane damage and lipid peroxidation due to UV exposure

	Hb (%)	MDA (µM)
Po(-)	24.465±0.630	158.000±1.732
Po(+)	34.035±0.106	200.800±1.532
P1	32.405±0.137	198.600±1.140
P2	20.391±0.207	183.400±1.673
P3	29.414±3.562	177.000±0.707

Based on the normality test, p=0.04 ($p<0.05$) for %Hb and p=0.03 ($p<0.05$) for MDA levels. The results of this test indicate that %Hb and MDA have abnormal data, so proceed with the Kruskal Wallis test.

The results of the Kruskal Wallis test showed that the administration of methanol extract of Sungkai leaves can inhibit erythrocyte membrane damage through inhibition of erythrocyte membrane lipid peroxidation.

UV exposure triggers the formation of reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radicals, and peroxides, which can attack the phospholipids of the erythrocyte membrane, causing lipid peroxidation, loss of membrane fluidity, and ultimately hemolysis (Grishchenko *et al*., 2020; Fini *et al*., 2022). D-limonene which is a

monoterpene compound, has strong antioxidant activity due to its structure containing conjugated double bonds, capable of capturing ROS before they attack the membrane (Rahman *et al*., 2022; Sunil *et al*., 2024). Oleic acid and 12-cis-octadecadienoate, as unsaturated fatty acids, are able to integrate into the membrane lipid layer and maintain the flexibility and stability of the membrane structure from oxidative damage, while playing a role in regulating the anti-inflammatory lipid balance (Li *et al.*, 2021; Alba *et al*., 2023; Doppelmayer *et al*., 2025).

In addition, Glycidyl(Z)-9-heptadecanoate, which contains epoxide groups, can interact with oxygen reactive groups and reduce damage to proteins or phospholipids in the membrane. Tridecanoic acid, although a saturated fatty acid, contributes to the bilayer structure of cell membranes and reduces susceptibility to oxidation (Yuan *et al*., 2022; Sonnen *et al*., 2025). Compounds such as 4-Methoxy-3-(trimethylsilyl)methyl-1-octene may play an additional protective role through a stabilizing physical effect or as a weak radical scavenger thanks to its electron-donating methoxy group (Kachadourian *et al*., 2012; Mollinedo *et al*, 2020).

The content of lipophilic compounds and antioxidants in the methanol extract of sungkai leaves supports the protection of erythrocyte membranes from disintegration due to UV exposure, both through neutralization of ROS and stabilization of membrane lipid structures.

IV. CONCLUSION

GC-MS analysis results show that sungkai leaf methanol extract contains compounds 4-Methoxy-3-trimethylsilyl)methyl-1-octene, D-Limonen, Tridecanoic acid, 12-cis-octadecadienoate, 13-Octadecanoic acid, methyl ester, Oleic acid, and Glycidyl(Z)-9-heptadecanoate which can reduce erythrocyte damage due to UV exposure.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this research.

ACKNOWLEDGEMENT

The authors would like to thank the Rector of Lambung Mangkurat University for providing PDWM research funds with contract No.1728/UN8/LT/2025.

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