Metabolite Profiling of Potential Fraction from Ethyl Acetate Extract of Ziziphus mauritiana Leaves by LC-MS/MS Analysis

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In Indonesia, the treatment with leaves as a traditional medicine is still firmly integrated into the community to overcome various health problems experienced, one of which is the treatment with Bidara leaves (*Ziziphus mauritiana*). *Ziziphus mauritiana* belongs to family of *Rhamnaceae, and it is generally considered a potential source of antioxidant and cholesterol lowering.* This study aimed to examine phytochemical characterization, antioxidant potential, and cholesterol-lowering effects of ethyl acetate fraction derived from *Ziziphus mauritiana*. Extraction was accomplished using ethyl acetate, and the resultant extract was fractionated through chromatography with a blend of n-hexane and ethyl acetate as the eluent. Antioxidant assay was conducted using the scavenging radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) methods, while cholesterol-lowering activity was assessed with the Lieberman-Burchard method. Additionally, phytochemical profiling of potential fractions was determined using LC-MS/MS. The results showed that fraction ethyl acetate *Ziziphus* (EAZ) fraction 09 had the highest DPPH radical scavenging with % inhibitions of 30.524 ± 0.095 µg/mL. Based on LC-MS/MS analysis, fraction EAZ.09 contained five main compounds and EC_{50} value of cholesterol-lowering activity was 132.115 µg/mL. In conclusion, fraction EAZ.09 from *Z. mauritiana* leaves had significant potential based on the chemical composition, in-vitro antioxidant activity, and cholesterol-lowering effects.

Keywords

Zizipus mauritiana, Bidara, Antioxidant, cholesterol-lowering activity

RESEARCH PAPER

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Abstract

In Indonesia, the treatment with leaves as a traditional medicine is still firmly integrated into the community to overcome various health problems experienced, one of which is the treatment with Bidara leaves (*Ziziphus mauritiana*). *Ziziphus mauritiana* belongs to family of *Rhamnaceae, and it is generally considered a potential source of antioxidant and cholesterol lowering*. This study aimed to examine phytochemical characterization, antioxidant potential, and cholesterol-lowering effects of ethyl acetate fraction derived from *Ziziphus mauritiana*. Extraction was accomplished using ethyl acetate, and the resultant extract was fractionated through chromatography with a blend of n-hexane and ethyl acetate as the eluent. Antioxidant assay was conducted using the scavenging radical 2,2- diphenyl-1-picrylhydrazyl (DPPH) methods, while cholesterol-lowering activity was assessed with the Lieberman-Burchard method. Additionally, phytochemical profiling of potential fractions was determined using LC-MS/MS. The results showed that fraction ethyl acetate *Ziziphus* (EAZ) fraction 09 had the highest DPPH radical scavenging with % inhibitions of 30.524 ± 0.095 μg/mL. Based on LC-MS/MS analysis, fraction EAZ.09 contained five main compounds and EC_{50} value of cholesterol-lowering activity was 132.115 μg/mL. In conclusion, fraction EAZ.09 from *Z. mauritiana* leaves had significant potential based on the chemical composition, in-vitro antioxidant activity, and cholesterol-lowering effects.

Keywords: Bidara, Antioxidant, Cholesterol-lowering activity, DPPH, *Ziziphus mauritiana*

1. Introduction

Natural medicines obtained from leaves of plants are considered a source for drug discovery [1]. Conventionally, the process of identifying lead compounds entails isolating and purifying each from plant extracts [2]. The isolation and purification of secondary metabolites is a complex and inefficient process, often leading to a low quantitative recovery. Furthermore, analyzing profiles and isolating bioactive compounds offer a comprehensive understanding of the dominant compound contents to guide the activity of separation methods [3]. In this context, LC-MS/MS method functions as a tool for analyzing numerous metabolites from fractions, allowing for the prediction of metabolic pathways. It is widely used for metabolite analysis due to the ability to access numerous integrated library resources in computer programming software [4].

An example of plant with potential medicinal utility is *Ziziphus mauritiana*, commonly known as bidara [5,6] and belongs to the *Rhamnaceae* family [7]. *Ziziphus* genus consists of about 40 species of spiny shrubs located in warm temperate and subtropical areas worldwide [8]. The broader family comprises 50−60 genera and roughly 870−900 species [9]. In traditional Chinese medicine, *Z. mauritiana* leaves are commonly used to alleviate various conditions including bronchitis, urinary problems, indigestion, pharyngitis, skin infections, fever, liver complaints, anemia, obesity, diabetes, diarrhea, and

Received 21 April 2024; revised 6 September 2024; accepted 11 September 2024.
Available online 5 November 2024

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https://doi.org/10.33640/2405-609X.3371

insomnia [10,11]. The plant is located in Cilegon region, specifically in Cimerak and phytochemical screening of leaves extract indicates the presence of alkaloids, flavonoids, triterpenoids, saponins, proteins, and lipids [12,13].

Z. mauritiana leaves have been investigated for the biological activities. Several studies focusing on leaf extract showed diverse bioactivities, including antioxidant properties [14], as well as antibacterial activity [15], cholesterol-lowering [16], antimicrobial [17], hepatotoxicity, anti-diabetic [18], and cytotoxicity potential [19]. Therefore, this study aimed to extract and isolate bioactive compounds from *Z. mauritiana* using chromatography methods. The bioactive fraction was subjected to LC-MS/MS analysis to identify chemical composition. In vitro antioxidant and cholesterol-lowering properties of fractions were also evaluated.

2. Material and methods

This study used a quantitative method, with 2,2-Diphenyl-1-Picrylhydrazil (DPPH) and cholesterol obtained from Sigma Aldrich, Inc. USA. Ethyl acetate p.a (pro analysis), *n*-hexane (pro analysis), H_2SO_4 (pro analysis), silica gel G60, and acetic anhydride (pro analysis) were acquired from Merck. Additionally, *Z. mauritiana* leaves were collected from Cilegon region, Cimerak Village, Banten Province, Indonesia.

2.1. Sample preparation

Z. mauritiana leaves were identified by an expert from the Herbarium Laboratory at Universitas Gadjah Mada in Yogyakarta, Indonesia with registration number 00685/S.TB./VII/2024. The samples were dried naturally under ambient conditions before being finely ground into fragments of roughly 0.5 cm in diameter. Subsequently, 500 g leaves were macerated with 1.5 L ethyl acetate for 24 h. Extraction was repeated three times successively. Ethyl acetate extract was concentrated at 50 °C using a rotary vacuum evaporator, yielding a solid mass of 8.7 g with a water bath.

2.2. Sample fractionation

Ethyl acetate extract was prepared for fractionation using column chromatography, following the procedure outlined in Musa et al. [20] with some modifications. The 8.7 g ethyl acetate extract from *Z. mauritiana* was subjected to chromatography on 87 g silica gel 70–320 mesh, using an n-hexane/ethyl acetate gradient elution (9/1 to 1/9) as eluting solvents. This process yielded 10 fractions namely EAZ.01-EAZ.10. Thin Layer Chromatography (TLC) was used to analyze the spots of fractions. Subsequently, EAZ.01-EAZ.10 were evaporated to obtain the final mass of all fractions. Free radical scavenging assay was performed according to inhibition percentage. Fraction with the highest inhibition percentage in DPPH was then analyzed using LC-MS/MS and tested for cholesterol-lowering activity assay.

2.3. Free radical scavenging assay

Antioxidant radical scavenging activity assay was conducted using DPPH (2,2-diphenylpicrylhydrazyl) following a modified method described by [21,22]. About 10 mg/L leaves fractions (EAZ.01-EAZ.10) were prepared with methanol pro analysis as a solvent. Each fraction EAZ.01-EAZ.10 was prepared at a concentration of 50 μg/mL, then 2.4 mL of the respective solution was mixed with 600 μL of 0.15 mM DPPH in a tube. The combination was vigorously mixed using a vortex mixer and allowed to stand without disturbance in a dark room at room temperature for 30 min [23]. Absorbance measurements were taken at a wavelength of 517 nm using UV spectrophotometer, with methanol as the reference blank [24]. The blank solution consisted of methanol and DPPH. The percentage of DPPH free radical inhibition was determined using the formula: % inhibition = (A_blank - A_sample)/A_blank x 100, where A_blank denotes the absorbance of the control reaction, and A_sample denotes the absorbance of the solutions containing the sample fractions [25,26].

2.4. LC-MS/MS analysis

LC-MS/MS analysis used a Waters Acquity UPLC I-Class system coupled with a Xevo G2-X2 Quadrupole Time of Flight (QToF) mass spectrometer. This study adhered to the Standard Operational Procedure for LC-MS/MS to create compound profiles of fraction samples. Subsequently, all LC-MS data were processed, peaks were selected, and analysis was conducted using the UNIFI informatics platform [27].

2.5. Cholesterol-lowering activity assay

The Liebermann-Burchard test was used to measure cholesterol-lowering activity, following Musa et al. [20] with slight modifications. Absorbance was compared with a blank, namely cholesterol solution and acetic anhydride to determine reductions in

cholesterol-lowering fraction. A stock cholesterol solution was dissolved in 50 mg cholesterol salt in 50 mL ethanol (1000 ppm). The samples were prepared at concentrations 50, 50, 100, 150, 200, and 250 μg/mL. Solutions without a sample were used as controls including cholesterol, acetic acid, and H_2SO_4. A mixture of 5 mL sample, 2 mL cholesterol solution, 2 mL acetic anhydride, and 0.1 mL H_2SO_4 was prepared in a tube. After incubation in the dark for 25 min at room temperature, the absorbance was measured at 423 nm. Cholesterol-lowering activity was calculated using the formula: % cholesterol lowering = (A0 - Ab)/A0 x 100, where A0 is the absorbance of the blank (control), and Ab is the absorbance of the sample. EC_{50} value was determined using Microsoft Excel software with a linear regression curve.

2.6. Statistical analysis

The data was presented as Mean ± SD, and statistical analysis was conducted using Microsoft Excel 2010. T-tests assumed equal variances, indicating significance levels as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. The % inhibition values for free radical scavenging and EC_{50} for cholesterol-lowering activity were obtained from the dose-response curve.

3. Result and discussion

This study used ethyl acetate for extraction, a more polar solvent compared to n-hexane. Ethyl acetate is often selected as a solvent for maceration due to the numerous advantageous properties. These include good solubility for a wide range of compounds, comprising both polar and non-polar substances [28]. The characteristics make ethyl acetate ideal for extracting various compounds from plant material through maceration [29]. A previous study indicated that among different solvents including n-hexane and methanol used for extraction, ethyl acetate extract of *Z. mauritiana* leaves had the highest antioxidant activity in scavenging DPPH free radicals. The yield was 8.7 g, accounting for 1.74 % of the starting material.

3.1. Fractionation of ethyl acetate extract

Ethyl acetate extract of *Z. mauritiana* was fractionated using column chromatography selected due to the effectiveness in separating mixtures based on differences in polarity, size, and other physical or chemical properties of the compounds present [30]. Column chromatography offers high resolution and separation power, isolating closely related compounds with varying polarities or molecular sizes [31]. This method is particularly useful for complex mixtures where other methods may not provide sufficient resolution. Furthermore, column chromatography enables the collection of individual fractions as the sample elutes from the column, allowing for the isolation of specific or groups of compounds from the mixture [32]. Using 8.7 g ethyl acetate extract, 10 fractions (EAZ.01-EAZ.10) were obtained, with the masses shown in Table 1. Subsequently, all fractions were evaluated for the ability to scavenge free radicals using DPPH method.

3.2. Free radical scavenging assay

DPPH is commonly used to assess antioxidant properties and free radical scavenging ability of fractions. The stable free radical can be stored for extended periods without substantial degradation, ensuring consistent and reliable results over time [33,34]. Each fraction (EAZ.01-EAZ.10) was diluted to a 50 μg/mL concentration using methanol as the solvent. DPPH was prepared at a concentration of 0.15 mM, with absorbance levels ranging from 6.00 to 7.00. The outcomes of the DPPH free radical scavenging assay for fractions EAZ.01-EAZ.10 are presented in Table 1 and Fig. 1.

The highest % inhibition of DPPH radical scavenging was produced by EAZ.09, with a value of 30.524 ± 0.095 ppm. This was indicated by a color change in the solution from purple to yellow, corresponding to a decrease in absorbance at 517 nm after a 30 min incubation period. The efficacy of scavenging free radicals, as indicated by the % inhibition value, was as follows: EAZ.09 > EAZ.07 > EAZ.08 > EAZ.06 > EAZ.04 > EAZ.02 > EAZ.03 > EAZ.10 > EAZ.05 > EAZ.01. Meanwhile, the lowest % inhibition was shown in EAZ.01. The results were

Table 1. % Inhibition in DPPH free radical scavenging of EAZ.01-EAZ.10 fractions from ethyl acetate extract of Z. mauritiana.

Fractions	Mass (g)	% DPPH radical scavenging replications			Mean ± SD (ppm)
		1*	2**	3**	
EAZ.01	0.43	3.306	3.802	3.471	3.526 ± 0.252
EAZ.02	0.88	11.405	11.570	11.405	11.460 ± 0.095
EAZ.03	0.35	10.909	10.744	10.744	10.799 ± 0.095
EAZ.04	0.23	11.736	11.736	11.570	11.681 ± 0.095
EAZ.05	0.28	5.289	5.289	5.619	5.399 ± 0.190
EAZ.06	0.11	15.372	15.207	15.537	15.372 ± 0.165
EAZ.07	0.14	20.826	20.661	20.611	20.699 ± 0.112
EAZ.08	0.23	18.347	18.512	18.347	18.402 ± 0.095
EAZ.09	0.07	30.413	30.579	30.579	30.524 ± 0.095
EAZ.10	0.32	10.083	10.083	10.413	10.193 ± 0.190

The *, ** and *** mean replications.

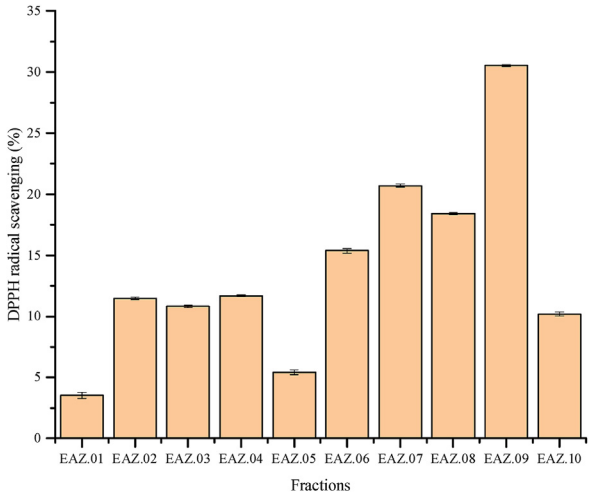

Fig. 1. DPPH free radical scavenging (%) of EAZ.01-EAZ.10 fractions.

consistent with other studies that reported ethyl acetate extract of *Z. mauritiana* leaves to be highly potent, demonstrating significant DPPH free radical scavenging activity. Yahia et al. [17] and Zain et al. [14] also found significant antioxidant potential, with IC50 values of 18.80 ± 1.63 μg/mL and 29.29 ± 1.02 μg/mL, respectively. Phytochemical screening of EAZ.09 showed the presence of phenolic, triterpenoid, and alkaloid compounds [35]. Antioxidant properties of phenolics were attributed to the hydroxyl groups, which scavenged free radicals [36,37]. EAZ.09 demonstrated great potential as a source of antioxidant compounds.

3.3. Cholesterol-lowering activity

Cholesterol-lowering activity of EAZ.09 is presented in Table 2. The Liebermann-Burchard method was used to assess cholesterol-lowering effects of EAZ.09. Cholesterol, with the molecular formula $C_{27}H_{45}OH$, is a steroid containing an alcohol functional group and commonly found in oils, milk, as well as animal fats. Elevated cholesterol levels in the bloodstream can lead to the formation of crystals or plaques that narrow and obstruct blood vessels. *Z. mauritiana* harbors various chemical constituents including flavonoids, alkaloids, terpenoids, phenolics, saponins, and tannins [19]. Among the many health-promoting attributes of *Z. mauritiana* leaf extract is the capacity to reduce cholesterol levels in the body.

Cholesterol-lowering activity of EAZ.09 was evidenced by EC_{50} value of 132.115 μg/mL. Phytochemical screening showed the presence of phenolic, triterpenoid, and alkaloid compounds. According to various studies, phenolic and triterpenoid compounds can effectively reduce cholesterol levels. Musa et al. [20] also reported that triterpenoid acid could reduce cholesterol levels.

3.4. LC-MS/MS analysis

To analyze secondary metabolites and corroborate the results of phytochemical screening, compound identification was performed using LS-MS/MS. The results in Table 3 showed the successful identification of several compounds such as 2-mono palmitin (1), 3β,16α-Dihydroxy-lanosta-8,24-dien-21-oic acid (2), and digiprolactone (3), along with two unidentified compounds. The structures of the compounds from EAZ.09 are depicted in Fig. 2.

Fig. 3 shows the fragmentation pattern of peak one from Table 3, eluted at 3.64 min, and corresponds to the structure of digiprolactone [38]. The fragments consist of m/z 197.1170 (observed, [M+H]+) and MSn fragmentation (m/z 179.1065).

Table 2. Cholesterol-lowering level of fraction EAZ.09 from Z. mauritiana *ethyl acetate extract.*

Concentrations	% lowering replications			Mean ± SD	Regression linear	EC_{50} (ppm)
	1*	2**	3**			
50	12.917	12.427	12.917	12.754 ± 0.28		
100	37.243	35.526	37.243	36.671 ± 0.99	$Y = 0.3934 \times 1.9742$	
150	64.269	64.035	64.223	64.176 ± 0.12	$R^2 = 0.9471$	132.115
200	84.269	84.356	84.269	84.298 ± 0.05		
250	87.243	87.390	87.243	87.292 ± 0.08		

The *, ** and *** mean replications.

Table 3. Compounds of EAZ.09 based on LC-MS/MS analysis data.

Compounds name	Formula	Observed m/z	Neutral mass (Da)	Rt (min)	Mass error (mDa)
2-monopalmitin	$C_{19}H_{38}O_4$	331.2844	330.27701	10.02	0.1
3β,16α-dihydroxy-lanosta-8,24-dien-21-oic acid	$C_{30}H_{48}O_4$	473.3623	472.35526	7.58	−0.3
digiprolactone	$C_{11}H_{16}O_3$	197.1170	196.10994	3.64	−0.3
Unknown compound	$C_{37}H_{40}N_4O_7$	653.2990	652.28970	10.91	2.0
Unknown compound	$C_7H_{10}O_4$	159.0652	158.05791	2.21	0.1

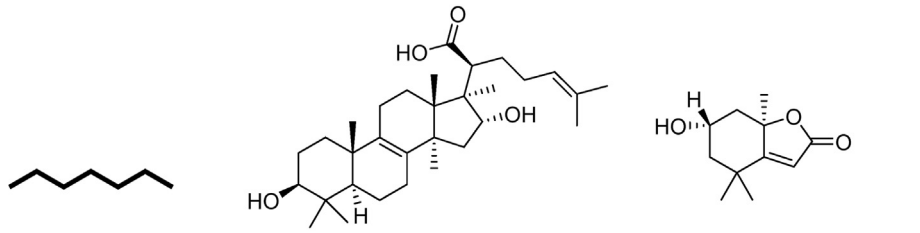

Fig. 2. Compound structures of EAZ.09 from ethyl acetate extract of Z. mauritiana *leaves.*

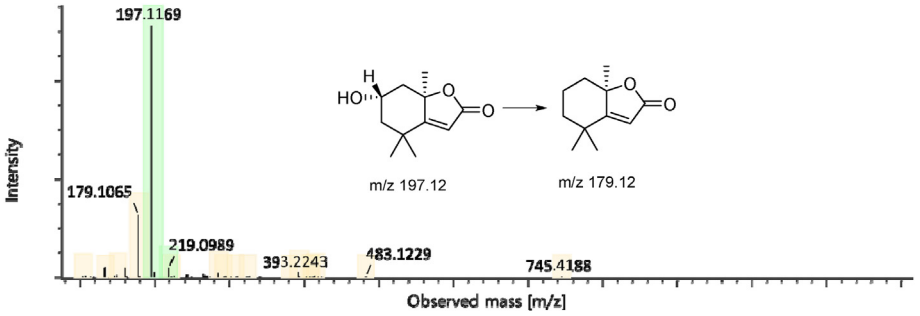

Fig. 3. The ESI-MSn fragmentation pattern of the identified compound digiprolactone from EAZ.09.

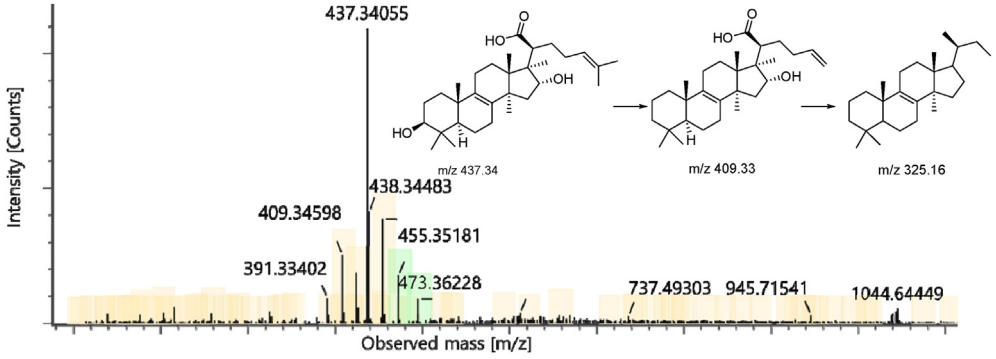

Fig. 4. The ESI-MSn fragmentation pattern of the identified compound 3β,16α-dihydroxy-lanosta-8,24-dien-21-oic acid from EAZ.09.

The m/z 197.1170 fragment was attributed to the molecular weight of digiprolactone plus the atomic weight of H or $[M+H]^+$. Peak number 2, eluted at 7.58 min and had a molecular weight of the neutral mass at m/z 473.3623, corresponded to 3β,16α-Dihydroxy-lanosta-8,24- dien-21- oic acid [39]. The structural conformity was supported by the fragmentation pattern in Fig. 4.

4. Conclusions

In conclusion, chemical profile, in-vitro antioxidant, and cholesterol-lowering properties of EAZ.09 from *Z. mauritiana* leaves demonstrated great potential, suggesting a promising nutraceutical application. These effects were attributed to compounds such as 3β,16α-Dihydroxy-lanosta-8,24-dien-21-oic acid, and digiprolactone. The % inhibition of DPPH by fraction EAZ 0.09 indicated a high level of antioxidant activity.

Ethics information

None.

Funding

The research didn't have a funding.

Acknowledgment

The authors are grateful to Mrs. Sofa Fajriah in the National Research and Innovation Agency (BRIN) Indonesia for LC-MS/MS analysis.

References

[1] A.N. Tavanappanavar, S.I. Mulla, C. Shekhar Seth, Z.K. Bagewadi, M. Rahamathulla, M. Ahmed, S. Farhana, Phytochemical analysis, GC–MS profile and determination of antibacterial, antifungal, anti-inflammatory, antioxidant activities of peel and seeds extracts (chloroform and ethyl acetate) of Tamarindus indica L, Saudi J. Biol. Sci. 31 (2024) 1–9, https://doi.org/10.1016/j.sjbs.2023.103878.

[2] F. Ali, Z. Saddiqe, M. Shahzad, A. Rafi, M. Javed, F. ul Haq, S. Saleem, S. Kusar, Crotalaria medicaginea Lamk.: an unexplored source of anticancer, antimicrobial and antioxidant agents, Eur. J. Integr. Med. 58 (2023) 1–11, https://doi.org/10.1016/j.eujim.2023.102226.

[3] H. Rao, I. Rao, L. Saeed, H.Y. Aati, S. Aati, M. Zeeshan, K. Khan, Phytochemical analysis and bioactivity assessment of five medicinal plants from Pakistan: exploring polyphenol contents, antioxidant potential, and antibacterial activities, Saudi J. Biol. Sci. 30 (2023) 1–11, https://doi.org/10.1016/j.sjbs.2023.103783.

[4] I. Sahidin, N. Nohong, M.A. Manggau, Wahyuni Arfan, I. Meylani, M.H. Malaka, N.S. Rahmatika, A.W.M. Yodha, N.U. E. Masrik, A. Kamaluddin, A. Sundowo, S. Fajriah, R. Asasutjarit, A. Fristiohady, R. Maryanti, N.I. Rahayu, M. Muktiarni, Phytochemical profile and biological activities of ethylacetate extract of peanut (Arachis hypogaea L.) stems: in-vitro and in-silico studies with bibliometric analysis, indones, J. Sci. Technol. 8 (2023) 217–242, https://doi.org/10.17509/ijost.v8i2.54822.

[5] M.K.M. Akanda, A.H.M.N. Hasan, Characterization of pharmacological properties of methanolic seed and stem bark extracts of Ziziphus mauritiana (BAU Kul) using in-vitro and in-vivo animal (Swiss albino male mice) model, Clin. Phytosci. 7 (8) (2021) 1–13, https://doi.org/10.1186/s40816-020-00246-0.

[6] A. Rahman, M.H. Harunsani, A.L. Tan, N. Ahmad, M.M. Khan, Antioxidant and antibacterial studies of phytogenic fabricated ZnO using aqueous leaf extract of Ziziphus mauritiana Lam, Chem. Pap. 75 (2021) 3295–3308, https://doi.org/10.1007/s11696-021-01553-7.

[7] M. Asimuddin, M.R. Shaik, N. Fathima, M.S. Afreen, S.F. Adil, M.R.H. Siddiqui, K. Jamil, M. Khan, Study of antibacterial properties of Ziziphus mauritiana based green synthesized silver nanoparticles against various bacterial strains, Sustain Times 12 (1484) (2020) 1–14, https://doi.org/10.3390/su12041484.

[8] A. Amin, A. El Asely, A.S. Abd El-Naby, F. Samir, A. El-Ashram, R. Sudhakaran, M.A.O. Dawood, Growth performance, intestinal histomorphology and growth-related gene expression in response to dietary Ziziphus mauritiana in Nile tilapia (Oreochromis niloticus), Aquaculture 512 (2019) 1–9, https://doi.org/10.1016/j.aquaculture.2019.734301.

[9] D. Dahiru, O. Obidoa, Evaluation of the antioxidant effects of Ziziphus mauritiana lam. leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver, African J. Tradit. Complement Altern. Med. 5 (2008) 39–45, https://doi.org/10.4314/ajtcam.v5i1.31254.

[10] E.N.H. Youl, C.A.P. Ouédraogo, M. Gambo, M. Ouédraogo, M. Kiendrebéogo, A. Traoré, I.P. Guissou, Antioxidant activity of crude ethanolic extract and fractions of Ziziphus mauritiana Lam. (Rhamnaceae) leaves from Burkina Faso, J. Basic Clin. Physiol. Pharmacol. 30 (2019) 1–9, https://doi.org/10.1515/jbcpp-2017-0176.

[11] A. Febriza, S. Faradiana, M.F. Abdullah, Antibacterial effects of Ziziphus mauritiana (lam) leaf extract against Vibrio cholerae, Herb-med J Terbit Berk Ilm Herbal, Kedokt dan Kesehat 5 (2022) 9–13, https://doi.org/10.30595/hmj.v5i3.14307.

[12] F.N. Jailani, U.H. Zaidan, M.B. Rahim, S.S. Gani, M. Halmi, Evaluation of constituents and physicochemical properties of Malaysian underutilized Ziziphus mauritiana (bidara) for nutraceutical potential, Int. J. Fruit Sci. 20 (3) (2020) 394–402, https://doi.org/10.1080/15538362.2019.1641458.

[13] A. Nikmatullah, I. Nairfana, S.M. Dewi, M. Sarjan, Morphological diversity of Indian jujube (Ziziphus mauritiana) in sumbawa island, west nusa tenggara, Indonesia, Biodiversitas 24 (2023) 4597–4608, https://doi.org/10.13057/biodiv/d240842.

[14] P. Jain, A. Haque, T. Islam, M.A. Alam, H.M. Reza, Comparative evaluation of Ziziphus mauritiana leaf extracts for phenolic content, antioxidant and antibacterial activities, J. Herbs, Spices, Med. Plants 25 (2019) 236–258, https://doi.org/10.1080/10496475.2019.1600627.

[15] E.A. Nurrahma, Antibacterial activity of bidara leaves (Ziziphus mauritiana l.) ethanol extract against some test bacteria, J Microbiol Sci 2 (2) (2022) 38–47, https://doi.org/10.56711/jms.v2i2.867.

[16] L. Rosalina, D. Mahendika, M.M.A.Z. Asyura, S.A. Aziz, T. Djafar, Utilization of Ziziphus mauritiana leaves for lowering cholesterol level, J. Penelit Pendidik IPA 9 (2023) 737–741, https://doi.org/10.29303/jppipa.v9i9.4268.

[17] Y. Yahia, M.A. Benabderrahim, N. Tlili, M. Bagues, K. Nagaz, Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two Ziziphus Mill. species, PLoS One 15 (2020) 1–16, https://doi.org/10.1371/journal.pone.0232599.

[18] K. Dubey, R. Dubey, R.A. Gupta, A.K. Gupta, Anti-diabetic and antioxidant potential of saponin extract of leaves of Ziziphus mauritiana, J. Drug Deliv. Ther. 9 (2019) 75–77. http://jddtonline.info.

[19] T.A. Owolarafe, K. Salawu, G.O. Ihegboro, C.J. Ononamadu, A.J. Alhassan, A.M. Wudil, Investigation of cytotoxicity potential of different extracts of Ziziphus mauritiana (Lam) leaf Allium cepa model, Toxicol. Rep. 7 (2020) 816–821, https://doi.org/10.1016/j.toxrep.2020.06.010.

[20] W.J.A. Musa, B. Situmeang, J. Sianturi, Anti-cholesterol triterpenoid acids from saurauia vulcani korth. (Actinidiaceae), Int. J. Food Prop. 22 (2019) 1439–1444, https://doi.org/10.1080/10942912.2019.1650762.

[21] M.F. Aldayel, Potential antibacterial and antioxidant inhibitory activities of Silybum marianum mediated biosynthesised He-Ne laser, Saudi J. Biol. Sci. 30 (2023) 1–11, https://doi.org/10.1016/j.sjbs.2023.103795.

[22] M.D. Astuti, K. Rosyidah, D. Umaningrum, R. Ardiyanti, H.J. A. Rasyied, A.P. Azzahra, The antioxidant activity and total phenolic content of sonneratia ovata back, Malaysian J. Fundam Appl. Sci. 19 (2023) 215–218, https://doi.org/10.11113/mjfas.v19n2.2789.

[23] B. Ludwaba, M.O. Jimoh, C.P. Laubscher, F. Nchu, Insecticidal and antioxidant potential of volatile compounds and nanoparticles from Tulbaghia violacea Harv. inoculated with endophytic fungus Beauveria bassiana (Bals.-Criv.) Vuill, South African, J. Bot. Le 165 (2024) 246–256, https://doi.org/10.1016/j.sajb.2023.12.028.

[24] B. Chaima, D. Boutlelis, L.A. Touhami, T. Hajer, T. Ali, In vitro antioxidant, anti-inflammatory, and photoprotective activities of aqueous extract of the endemic plant Hammada scoparia L. from Algeria, Karbala Int. J. Mod. Sci. 9 (2023) 544–552, https://doi.org/10.33640/2405-609X.3310.

[25] F. Kirmani, Z. Saddiqe, S. Saleem, F. Ali, F. ul Haq, Phytochemical investigation and antibacterial activity of Curcuma longa against multi-drug resistant bacteria, South African J. Bot. 164 (2024) 137–145, https://doi.org/10.1016/j.sajb.2023.11.042.

[26] D.O. Nwude, P.M. Osamudiamen, S.M. Enessy, Phytochemical investigation of Mezoneuron Benthamianum Baill, isolation, in vitro antioxidant, alpha-amylase inhibition, and in silico modelling studies, South African J. Bot. 165 (2024) 526–537, https://doi.org/10.1016/j.sajb.2024.01.003.

[27] D. Sharma, V.P. Singh, N.K. Singh, A review on phytochemistry and pharmacology of medicinal as well as poisonous mushrooms, Mini-Rev. Med. Chem. 18 (2017) 1095–1109, https://doi.org/10.2174/1389557517666170927144119.

[28] T.H. Quang, N.X. Cuong, C. Van Minh, P. Van Kiem, New flavonoids from Baeckea frutescens and their antioxidant activity, Nat. Prod. Commun. 3 (2008) 755–758, https://doi.org/10.1177/1934578x0800300515.
[29] D.E. Rotimi, O.S. Adeyemi, Comparative evaluation of the antioxidant activity, trace elements, and phytochemical analysis of the extracts of unripe plantain whole fruit and pulp, Karbala Int. J. Mod. Sci. 9 (2023) 168–177, https://doi.org/10.33640/2405-609X.3290.
[30] M. Javed, R. Bibi, K. Nazir, S. Hussain, Phytochemistry of Ziziphus mauritiana; its antioxidant and antimicrobial potential, Adv. Life Sci. 9 (2022) 157–162.
[31] R.H. Kusriani, S.M. Rosandhy, E. Elfahmi, Luteolin, a flavonoid from syzygium myrtifolium walp, Curr. Res. Biosci. Biotechnol. 1 (2019) 31–33, https://doi.org/10.5614/crbb.2019.1.1/fkan4064.
[32] A.K. Mishra, K.N. Tiwari, R. Saini, J.K. Chaurasia, S.K. Mishra, Assessment of antioxidant potential in seed extracts of Nyctanthes arbor-tristis L. and phytochemical profiling by Gas Chromatography-Mass Spectrometry system, Brazilian J. Pharm. Sci. 58 (2022) 1–16, https://doi.org/10.1590/s2175-97902022e21180.
[33] S.Y. Kim, The antioxidant and anti-complementary activities of crude polysaccharides from trifoliate orange (poncirus trifoliate) seeds, Prev. Nutr. Food Sci. 28 (2023) 321–327, https://doi.org/10.3746/pnf.2023.28.3.321.
[34] W.J.A. Musa, N. Bialangi, A.K. Kilo, B. Situmeang, N.T. Susparini, I.D. Rusydi, Antioxidant, cholesterol lowering activity, and analysis of Caesalpinia bonducella seeds extract, Pharmacia 70 (2023) 97–103, https://doi.org/10.3897/pharmacia.70.e96817.
[35] V. Chaudhary, P. Katyal, J. Kaur, S. Bhatia, S. Singh, A.K. Poonia, A.K. Puniya, A. Raposo, S. Yoo, H. Han, H.A. Alturki, A. Kumar, Bioactive activity and safety analysis of Monascus red biopigment, Food Biosci. 57 (2024) 1–7, https://doi.org/10.1016/j.fbio.2023.103523.
[36] P. Kabré, L. Ouattara, Y. Sanou, R.J. Ouédraogo, P. Ouoba, A.A. Zanté, D. Zoungo, M.B. Somda, G.A. Ouédraogo, Comparative study of polyphenols, flavonoids content, antioxidant and antidiabetic activities of Lophira lanceolata Tiegh.ex Keay (Ochnaceae) extracts, Sci. African 22 (2023) 1–11, https://doi.org/10.1016/j.sciaf.2023.e01922.
[37] R. Alkowni, N. Jaradat, S. Fares, Total phenol, flavonoids, and tannin contents, antimicrobial, antioxidant, vital digestion enzymes inhibitory and cytotoxic activities of Verbascum fruticulosum, Eur. J. Integr. Med. 60 (2023) 1–12, https://doi.org/10.1016/j.eujim.2023.102256.
[38] S. Nur, H. Setiawan, M. Hanafi, B. Elya, Phytochemical composition, antioxidant, in vitro and in silico studies of active compounds of Curculigo latifolia extracts as promising elastase inhibitor, Saudi J. Biol. Sci. 30 (2023) 1–11, https://doi.org/10.1016/j.sjbs.2023.103716.
[39] G.Y. Lian, Q.M. Wang, P.M.K. Tang, S. Zhou, X.R. Huang, H. Y. Lan, Combination of asiatic acid and naringenin modulates NK cell anti-cancer immunity by rebalancing smad3/smad7 signaling, Mol. Ther. 26 (2018) 2255–2266, https://doi.org/10.1016/j.ymthe.2018.06.016.