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Antioxidant activity and GC-MS analysis of stem bark extract of Cingkam (*Bischofia javanica*) from North Sumatra, Indonesia

Hendro PRANOTO *, Ivan I. L. GAOL, and Meida NUGRAHALIA

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jalan Willem Iskandar Pasar V Medan, Indonesia.
*Corresponding author: H. Pranoto, E-mail: hendrop.unimed77@gmail.com

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Abstract. This study aimed to examine the antioxidant activity and identify bioactive compounds present in the stem bark extract of *Bischofia javanica*. Samples were collected from Beringin Park, Jenderal Sudirman Street, North Sumatra Province, Indonesia. The extraction method employed in this research was maceration, utilizing three different solvents. The antioxidant activity was evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay, while the identification of bioactive compounds was performed using GC-MS (Gas Chromatography-Mass Spectrometry) analysis. The results demonstrated varying levels of antioxidant activity in the methanol, ethyl acetate, and n-hexane extracts of *B. javanica* stem bark. The antioxidant activity was quantified by determining the IC_{50} value (inhibitory concentration at 50%). The IC_{50} values for the methanol, ethyl acetate, and n-hexane extracts were 31 μg/ml (strong), 467.09 μg/ml (weak), and 1521.69 μg/ml (weak), respectively. Furthermore, the study identified a total of 91 bioactive compounds, with 16, 34, and 41 compounds identified in the methanol, ethyl acetate, and n-hexane extracts, respectively. Based on the research findings, it can be concluded that the methanol extract of *B. javanica* stem bark exhibited potential as an antioxidant.

Keywords: bioactive compounds, methanol, ethyl acetate, n-hexane methanol, ethyl acetate, n-hexane.

Introduction

The antioxidant activity of a plant is often investigated as an initial step to assess its potential in treating various diseases such as diabetes, liver ailments, and cancer (Hiraganahalli et al. 2012, Osadebe et al. 2012). Communities in Indonesia widely favor the utilization of traditional medicinal plants due to their perceived safety and affordability (Isnawati et al. 2019, Widayati et al. 2021, Arifah et al. 2022, Humairani et al. 2023). One such potentially medicinal plant is *Bischofia javanica*. The traditional use of different parts of the plant, including its fruits, leaves, roots, seeds, wood, and stem bark, has been reported in previous studies (Rajbongshi et al. 2014). The Karo tribe in Indonesia has long used the stem bark of this plant as an antidiabetic remedy by boiling and consuming it (Nasution et al. 2018). Additionally, this plant has been reported to possess pharmacological activities including anti-leukemia, antimicrobial, and anti-inflammatory effects (Khan et al. 2001, Barla et al. 2010, Moghaddam et al. 2012).

Bischofia javanica is a native species to South Asia, Southeast Asia, Australia, and China, and grows in lowland areas up to an altitude of approximately 1500 m above sea level (Abe et al. 2020). It has a wide distribution, ranging from western India, southern Japan, eastern Australia, across the Pacific region to the Indonesian archipelago (Kundu et al. 2012). This plant belongs to the Euphorbiaceae family and is commonly known by different local names: 'singkam' and 'cingkam' in the Batak language,

'tingkeum' in Gayo, and 'gadog,' 'gintung,' 'kerinjing' in Javanese (Lubis & Nazwah 2024). In other countries, it is referred to as 'jitang' in Malaysia, 'tuai' in Sabah and the Philippines, 'toem' and 'pradu-som' in Thailand, 'khom fat' in Laos, and 'nhoi' in Vietnam (Prabowo 2019). The plant can attain a height of up to 40 m, with a trunk diameter ranging from 95 to 150 cm. It branches extensively, exhibiting an upright growth pattern. The wood is usually robust and hard, with a circular trunk shape that lacks knots. It falls under the category of perennial plants (Kundu et al. 2012). The outer bark of the trunk is scaly and varies in colour from reddish-brown to purplish. It exudes a clear red sap, which can be either watery or slightly gel-like (Rajbongshi et al. 2014). The leaves range in length from 4 to 8 inches and are 7 to 22 mm thick. They are elliptical with three lobes and taper to a pointed tip. The leaves are arranged circularly, supported by long petioles (3 - 8 inches) with finely serrated edges. The leaf veins are pinnate, and the upper surface of the leaf is glossy. The fruit does not dehisce and is spherical, latex-bearing, with a central diameter of 1.2 - 1.5 cm (Bhatnagar et al. 2023). When mature, it turns bluish-black (Bachheti et al. 2013).

Several studies have reported the antioxidant activity of *B. javanica* stem bark using various solvents, such as ethanol, ethyl acetate, n-hexane fraction, and methanol (Sutharson et al. 2009, Jambak et al. 2019, Ati et al. 2021). Furthermore, GC-MS analysis of *B. javanica* seeds revealed the presence of six essential fatty acids (Indra et al. 2013). However, the bioactive compounds of *B. javanica* have never been reported, where the identification of bioactive compounds from medicinal plants is crucial to estimate their pharmacological potential more specifically (Sasidharan et al. 2011, Anand et al. 2019). This study aims to evaluate the antioxidant activity of methanol, ethyl acetate, and n-hexane extracts using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method and identify bioactive compounds using GC-MS (Gas Chromatography-Mass Spectrometry) analysis.

Materials and methods

Plant collection and extraction preparation

The stem bark of *B. javanica* was collected in March 2021 from Beringin Park, Jenderal Sudirman Street, North Sumatra Province, Indonesia. Latitude and longitude coordinates are 3°34'35.1"N 98°40'10.0" E. Plant identification was based on Orwa et al. (2009).

Tree bark samples were taken during the dry season, from old plants that were 25 meters tall and bearing fruit. Select a healthy area of bark, then cut it into a circle and take 100 grams. The outer epidermis of the bark was cleaned and sliced into 2 cm pieces. Subsequently, it was dried in an oven at 40°C for five days and then dried under sunlight for two days. The dried powder was macerated using three different solvents, namely methanol, ethyl acetate, and n-hexane. The ratio of dried powder to solvent was 1:10. Maceration was carried out for eight days, followed by filtration and evaporation using a rotary evaporator.

Assay of free radical scavenging activity by the DPPH method

The DPPH method was performed based on Mbaebie et al. (2012) with slight modifications. A total of 9.875 mg of DPPH was dissolved in 50 mL of methanol. Standard stock solution (ascorbic acid) was prepared with varying concentrations of 2, 3, 4, 5, 6, and 7 μg/mL. Since the weight of the extract obtained from the three different solvents varied, sample stock solutions were prepared with the following concentration variations: 10, 20, 30, 40, 50, and 60 μg/mL (methanol extract); 300, 400, 500, 600, 700, and 800 μg/mL (ethyl acetate extract); 1000, 1250, 1500, 1750, 2000, and 2500 μg/mL (n-hexane extract).

To test the antioxidant capacity, 1600 μL of each standard solution, sample solution, and blank (methanol) were pipetted. Then, 400 μL of

the DPPH solution was added to each sample, and the mixture was homogenized and incubated at room temperature in the dark for 30 minutes. The absorbance of the solutions was measured at a wavelength of 517 nm. A calibration curve was constructed by plotting the percentage inhibition (y) against the concentration (x), yielding a regression equation (y = ax + b). The IC_{50} inhibitory concentration at 50% (inhibitory concentration at 50%) value was calculated using the formula ((50-b) / a).

GC-MS analysis

The analysis of bioactive compounds was conducted based on the methods described by Casuga et al. (2016) and Al-Owaisi et al. (2014) using an Agilent Technologies GC system, specifically the GC-7890B and MS-240 models. Spectroscopic analyses involve an electron ionization system using high-energy electrons (70 eV). Helium gas (99.995%) was used as the carrier gas at a 1 ml/minute flow rate. The initial temperature was set within the range of 50-150°C. A total of 1 µl of the extract solution was injected into the injector. Subsequently, the identification and characterization of bioactive compounds from the three extracts were performed based on the GC retention time. The mass spectra were matched against available standards in the National Institute of Standards and Technology (NIST) database.

Results

The chemical compositions of various extracts of *B. javanica* stem bark based on GC-MS analysis are presented in Tables 1, 2, and 3. The chromatograms of the stem bark extracts, as shown in Figures 1, 2, and 3, display the retention time (RT) and peak heights of the bioactive compounds present in the extracts. The methanol, ethyl acetate, and n-hexane extracts contained 16, 34, and 41 bioactive compounds.

Table 1. Biologically active chemical compounds of methanol stem bark extract from *Bischofia javanica.*

No.	Compound name	Molecular formula	RT (min)
1	Silanediol, dimethyl	$C_2H_8O_2Si$	3.27
2	3-Furaldehyde	$C_5H_4O_2$	5.064
3	Ethanol, 2-butoxy	$C_6H_{14}O_2$	6.748
4	1-Methylsulfanyl-1-hexen-3-one	$C_7H_{12}OS$	9.164
5	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	11.434
6	2-Amino-3-phenyl-6-nitroindole	$C_{14}H_{11}N_3O_2$	12.24
7	(6R,5'S)-6,5':7,5'- Dianhydro-5,6-dihydro-6,5'- dihydroxy-6-(hydroxymethyl)-2',3'-O-isopropylideneuridine isomer	$C_{13}H_{16}N_2O_7$	13.338
8	Benzothieno[2,3-c]quinolin-6(5H)one, 2- methoxy	$C_{16}H_{11}NO_2S$	19.781
9	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	24.284
10	2-(5-tert-Butyl-2-hydroxyphenyl)- 1 ,4-benzoquinone	$C_{16}H_{16}O_3$	29.043
11	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	38.672
12	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	47.678
13	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	49.801
14	Hexadecanoic acid	$C_{16}H_{32}O_2$	50.533
15	1-Undecyne	$C_{11}H_{20}$	52.364
16	Phenyl 4- [bis(ethoxycarbonyl)but-3-ynyl]- 2,3,4-trideoxy-.alpha.,L-glcero-pent-2-enopyranoside	$C_{21}H_{26}O_6$	57.672

Hexadecanoic acid methyl ester was found in the methanol, ethyl acetate, and n-hexane extracts. Hexadecanoic acid was present in the methanol and n-hexane extracts. 1-Undecanol was detected in the ethyl acetate and n-hexane extracts. Squalene was identified in the ethyl acetate and n-hexane extracts. Phthalic acid (2-propyl pentyl) ester was found in the ethyl acetate and n-hexane extracts.

The antioxidant activity data of the three extracts of *B. javanica* stem bark are presented in Table 3. The use of different solvents during the extraction process affects the antioxidant activity obtained (Table 4).

Table 2. Biologically active chemical compounds of ethyl acetate stem bark extract from *Bischofia javanica.*

No.	Compound name	Molecular formula	RT (min)
1	Benzene, methyl	C_7H_8	3.71
2	Butanoic acid, 1-methylpropyl ester	$C_8H_{16}O_2$	4.259
3	Methyl Laurate	C_8H_{10}	5.503
4	Unidentified C2-benzene	C_8H_{10}	5.723
5	Benzene, ethenyl	C_8H_8	6.309
6	Ethanol 2-butoxy	$C_6H_{14}O_2$	6.748
7	Ethane, 1,1,2,2-tetrachloro	$C_2H_2Cl_4$	6.931
8	7,7-Dimethyl Cycloheptatriene	C_9H_{12}	7.297
9	cis/trans-5-Phenyl-2- tetrahydrofurylmethyl 2′-pyridyl sulfide	$C_{16}H_{17}NOS$	9.164
10	x – ethyl – x – methyl –benzene	C_9H_{12}	9.677
11	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	9.933
12	Acetonyl decyl ether	$C_{13}H_{26}O_2$	14.07
13	3-(4-Nitrophenyl)quinazolin-4(3H)-one	$C_{14}H_9N_3O_3$	15.095
14	1-Heptadecene	$C_{17}H_{34}$	18.134
15	1-tridecanol	$C_{13}H_{28}O$	26.883
16	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	32.009
17	(1RS,2RS,4SR,5SR)-6- Oxabicyclo[3.1.0]hexane-2,4-diol	$C_5H_8O_3$	34.205
18	1-Undecanol	$C_{11}H_{24}O$	34.901
19	Tridecanoic acid	$C_{13}H_{26}O_2$	45.005
20	Neopentyl2,2dimethylpropanoate	$C_{10}H_{20}O_2$	46.469
21	Pentane, 3-bromo	$C_{10}H_{20}O_2$	46.799
22	Cyclononasiloxane,octadecamethyl	$C_{20}H_{28}N_2O_2$	47.677
23	1,2-Benzenedicarboxylic acid, bis(2methylpropyl) ester	$C_{12}H_{12}ClF_3O_2$	48.556
24	Pentadecane	$C_{15}H_{32}$	49.252
25	Methanesulfonic Acid, trifluoro-, 2,2dimethyl-1,3-propanediyl ester	$C_7H_{10}F_6O_6S_2$	49.654
26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	49.801
27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	50.57
28	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	52.876
29	Tritriacontane	$C_{33}H_{68}$	53.498
30	1-hexacosanol	$C_{26}H_{54}O$	55.439
31	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	56.793
32	Adipic acid, hexyl 2-propyl ester	$C_{15}H_{28}O_4$	58.038
33	Adipic acid, 3-hexyl nonyl ester	$C_{21}H_{40}O_4$	58.221
34	Squalene	$C_{30}H_{50}$	58.88

Table 3. Biologically active chemical compounds of n-hexane stem bark extract from *Bischofia javanica.*

No.	Compound name	Molecular formula	RT (min)
1	Cyclopentanol, 1-methyl	$C_6H_{12}O$	4.039
2	Benzene, ethyl	C_8H_{10}	5.467
3	p-Xylene	C_8H_{10}	5.686
4	Benzene, 1,3-dimethyl	C_8H_{10}	6.309
5	Pentanal, 2,2-dimethyl	$C_7H_{14}O$	7.371
6	N-Benzyl-N-(phenylethyl)-amine	$C_{15}H_{17}N$	8.213
7	Benzene, 1-ethyl-4-methyl	C9H12	9.164
8	Benzene, 1,2,3-trimethyl	C9H12	9.677
9	2-Ethylthio-2-ethoxy-3-oxo-N-phenylbutanamide	C14H19NO3S	9.933
10	unidentified C3-benzene	C9H12	10.775
11	Ethane, hexachloro	C_2Cl_6	12.789
12	Disulfide, dioctyl	$C_{16}H_{34}S_2$	14.033
13	1-Tridecene	$C_{13}H_{26}$	18.134
14	1-Decanol	$C_{10}H_{22}O$	26.883
15	1-Undecanol	$C_{11}H_{24}O$	34.901
16	Phenol, 2-(1-phenylethyl)	$C_{14}H_{14}O$	37.39
17	Nonadecane	$C_{19}H_{40}$	40.209
18	3-Octadecene	$C_{18}H_{36}$	46.177
19	Pentatriacontane	$C_{35}H_{72}$	46.469
20	Heptadecane	$C_{17}H_{36}$	46.799
21	1,2-Epoxy heptane	$C_7H_{14}O$	48.263
22	[(Butyl)-(4'-Chlorobenzyl)-(Methoxy)phosphine]	$C_{12}H_{18}ClO_2P$	48.556
23	Heptacosane	$C_{27}H_{56}$	49.105
24	Tricosane	$C_{23}H_{48}$	49.252
25	2-(2-Methyl phenyl)ethanol	C9H12O	49.398
26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	49.801
27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	50.533
28	1-Nonadecane	$C_{19}H_{38}$	50.936
29	Heneicosane	$C_{21}H_{44}$	52.4
30	1-Pentadecene	$C_{15}H_{28}$	52.876
31	Dihexylsulfide	$C_{12}H_{26}S$	55.146
32	Tetracosane	$C_{24}H_{50}$	55.475
33	Pentacosane	$C_{25}H_{52}$	56.317
34	Phthalic acid, (2-propylpentyl) ester	$C_{24}H_{38}O_4$	56.793
35	Hexacosane	$C_{26}H_{54}$	57.123
36	Tetracosane, 3-ethyl	$C_{26}H_{54}$	57.599
37	Octacosane	$C_{28}H_{58}$	57.892
38	3-Methyl hexacosane	$C_{27}H_{56}$	58.331
39	Squalene	$C_{30}H_{50}$	58.88
40	Celidoniol, deoxy	$C_{29}H_{60}$	59.319
41	Hentriacontane	$C_{31}H_{64}$	60.015

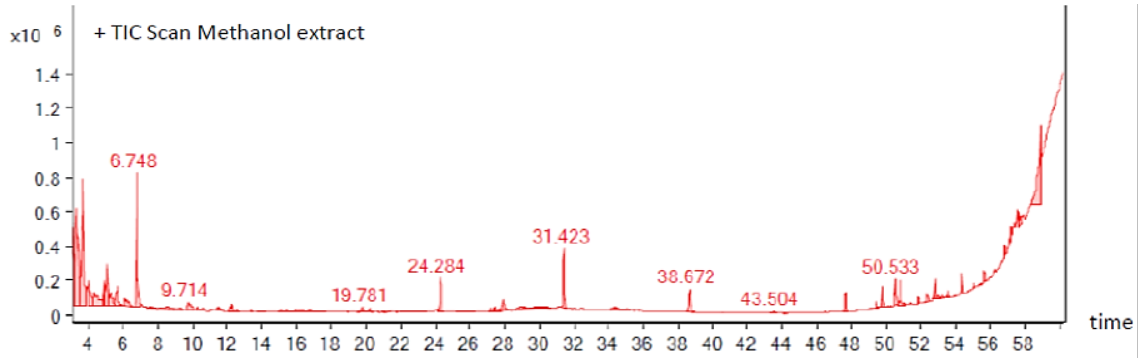

Figure 1. GC-MS chromatogram of the methanol extract of *Bischofia javanica* stem bark.

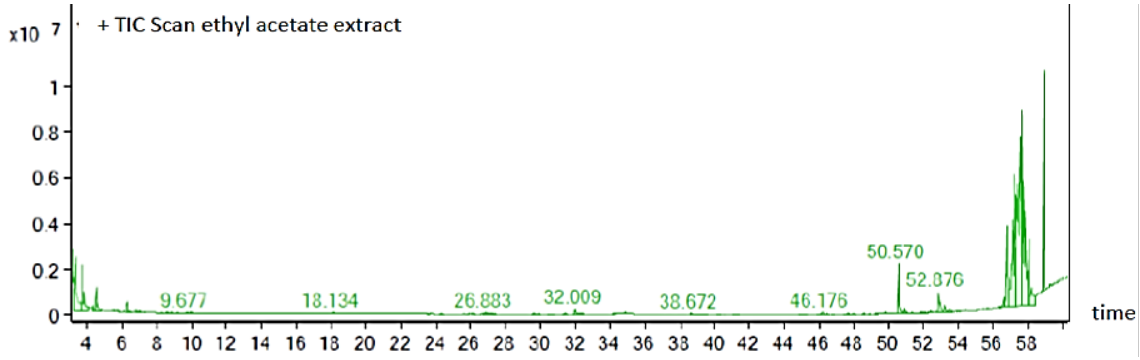

Figure 2. GC-MS chromatogram of ethyl acetate extract of *Bischofia javanica* stem bark.

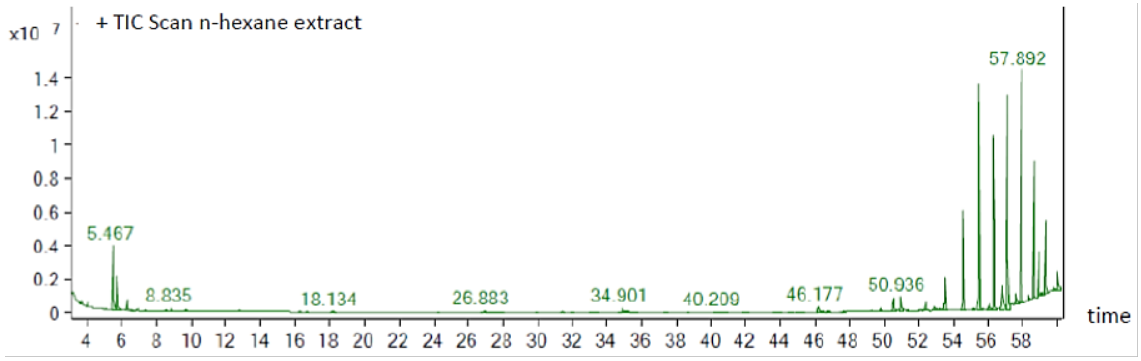

Figure 3. GC-MS chromatogram of n-hexane extract of *Bischofia javanica* stem bark.

Discussion

Several chemical compounds found in the stem bark extract have pharmacological activities. Cyclohexasiloxane and dodecamethyl have activities as lubricants and defoaming agents (Dinesh et al. 2018). Hexadecanoic acid methyl ester exhibits antioxidant, hypolipidemic, nematicidal, pesticide, and alpha-reductase

Table 4. IC_{50} values of the methanol, ethyl acetate, and n-hexane extracts of stem bark from *Bischofia javanica.*

No.	Extracts	IC_{50} (ppm)
1	Ascorbic acid	4.63
2	Methanol	31.00
3	Ethyl acetate	467.09
4	n-heksan	1521.69

inhibitor activities (Dinesh et al. 2018). n-Hexadecanoic acid, cyclooctasiloxane, hexadecamethyl, and cyclononasiloxane, octadecamethyl have pharmacological activities as antimicrobials (Ferdosi et al. 2021). Hexanoic acid and n-Hexadecaonoic acid have antimicrobial, antioxidant, anticancer, hypercholesterolemic, and antiulcerogenic activities (Kumar et al. 2010). Cyclotetrasiloxane, octamethyl, exhibits antifungal, antibacterial, and antioxidant activities (Ismail et al. 2020). 1-Heptadecene has insecticidal activity (Adebisi et al. 2019) and antioxidant activity (Hamidi et al. 2012). 1-tridecanol acts as a natural mosquito control agent (Tabanca et al. 2014) and possesses antibacterial activity (Kubo et al. 1993). Phenol, 2,4-bis(1,1-dimethylethyl) exhibits antifungal activity (Ren et al. 2019) and antimicrobial activity (Teresa et al. 2014). 1-Undecanol has flavor and perfumery activity (Selvamangai & Bhaskar 2012). Tridecanoic acid is a fatty acid, and Nonadecane shows potential as an antimicrobial and antioxidant (Banakar & Jayaraj 2005, Salem et al. 2014). 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester exhibits anti-proliferative activity (Kumar et al. 2021). Pentadecane inhibits the growth of *Leishmania infantum* in vitro (Bruno et al. 2015, Tewtrakul et al. 2005). 9,12-Octadecadienoic acid has anticancer, anti-inflammatory, and antiarthritis activities (Parthipan et al. 2015) as well as antimicrobial, anti-inflammatory, and antioxidant activities (Mathur et al. 2011). 1-hexacosanol acts as a larvicide by inhibiting acetylcholinesterase enzyme (Gade et al. 2017). 1-hexacosanol inhibits the growth of *Leishmania donovani* (Kavitha et al. 2009, Amin et al. 2017) and exhibits potential as an antioxidant and antimicrobial agent (Castilho et al. 2012).

Other compounds, such as squalene, have been found to exhibit various pharmacological activities. Squalene has been reported to reduce skin damage caused by UV radiation, lower LDL cholesterol levels, prevent cardiovascular diseases, and demonstrate antitumor and anticancer properties (Lozano-Grande et al. 2018). 2-Ethylthio-2-ethoxy-3-oxo-N-phenyl-butanamide has shown activity as an antitumor agent in nasopharyngeal carcinoma and serves as a neostigmine antidote (Vani et al. 2017). Disulfide, dioctyl have potential antibacterial effects (Ibeyaima et al. 2017, Joondan et al. 2020). 1-Tridecene has potential antioxidant activity (Sharma & Shaha 2014). 1-Decanol exhibits larvicidal potential (Ali et al. 2020) and antibacterial effects (Elgaali et al. 2002). 3-Octadecene shows potential as an antimicrobial agent (Balachandara et al. 2018) and possesses antibacterial, antioxidant, and anticancer properties (Gnanashree & Sirajudeen 2018). Pentatriacontane has potential as an antidiabetic and antioxidant compound (Laha & Paul 2019). Heptadecane exhibits anti-inflammatory effects (Kim et al. 2013) and antibacterial activity (Ozdemir et al. 2004). Heptacosane and Octacosane have potential antibacterial effects (Boukhebti et al. 2015, Khatua et al. 2016). Tricosane and Heneicosane show potential as antidiabetic agents (Asgarpanah & Kazemivash 2013, Savych et al. 2017). 1-Pentadecene exhibits anti-inflammatory effects (Wahrendorf & Wink 2006). Tetracosane demonstrates cytotoxic activity against cancer cells (Uddin et al. 2012). Pentacosane exhibits antifungal, antimicrobial, and antioxidant activities (Al-Amery & Al-Garaawi 2020). Hexacosane exhibits insecticidal activity (Poonsri et al. 2015). Celidoniol, deoxy or nonacosane have potential as antibacterial agents (Köse et al. 2016, Barretto & Vootla 2018) and possess anti-inflammatory properties (Zakaria et al. 2014). Hentriacontane exhibits anti-inflammatory effects (Khajuria et al. 2017) and shows antitumor activity (Takahashi et al. 1995). However, to determine the therapeutic benefits of the *B. javanica* stem bark extract, further isolation and pharmacological studies of each bioactive compound are required.

The antioxidant activity of the methanol, ethyl acetate, and n-hexane extracts, expressed as IC_{50} values, was found to be 31 ppm (very strong), 467.09 ppm (very weak), and 1521.69 ppm (very weak), respectively. This is likely due

to the polar bioactive compounds exhibiting better antioxidant activity compared to nonpolar and semipolar compounds. However, the antioxidant activity of the methanol extract of *B. javanica* stem bark was lower compared to that of vitamin C (ascorbic acid). The antioxidant activity of the methanol extract in this study was higher than that reported by Sutharson et al. (2009), while the antioxidant activity of the ethyl acetate extract was lower compared to the survey conducted by Ati et al. (2020).

Conclusions

The identification of bioactive compounds reveals that *B. javanica* has significant potential as a medicinal plant. The methanol extract exhibits higher antioxidant activity compared to the ethyl acetate and n-hexane extracts, but lower activity compared to ascorbic acid. Based on the research findings, it can be concluded that the stem bark of *B. javanica* can be utilized as a natural source of antioxidants. Further in vitro and in vivo studies, as well as the isolation of bioactive compounds, are necessary to determine their biological and pharmacological activities.

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