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Original Research Article

Evaluation of UV-Absorbing Capacity, Antioxidant and Antibacterial Activities of Natural Dyes Used in Indonesian Traditional Batik

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ABSTRACT

Batik has been part of Indonesian culture, where natural dyes are applied in textile production. The current study aimed to evaluate the health benefits of commonly used natural dyes of Batik; *Secang* (*Caesalpinia sappan* L.), *Telang* (*Clitoria ternatea* L.) and *Putri malu* (*Mimosa pudica* L.) based on their UV absorbing properties, antioxidant and antibacterial activity. UV absorbing properties was evaluated based on UV spectral data analysis. Antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, while the antibacterial activity was evaluated against against *Staphylococcus aureus* using the disc diffusion assay. *Secang* was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to identify potentially bioactive compounds. The UV spectral data analysis indicated that *Secang* dye absorbs dangerous UV-B radiation at 280 - 315 nm and possesses the highest antioxidant activities with an IC_{50} value of 10.76 µg/mL. In addition, *Secang* dye exhibited antibacterial activity against *Staphylococcus aureus* with inhibition zone diameter of 19.45 ± 1.05 mm at a concentration of 30 mg/mL. GC-MS analysis identified 35 compounds in *Secang*, in which several common antibacterial active compounds, including glycol congener pentaethylene glycol, pyridine, and naphthalene were present. In summary, natural Batik dyes are highly valued not only for their artistic value, but also for their potential health benefits.

***Keywords*:** Batik, *Caesalpinia sappan*, *Clitoria ternatea*, *Mimosa pudica*, Antioxidant, Antibacterial.

Introduction

In early human history, mankind has relied on natural dyes for dying textiles. However, since the 19th century, synthetic dyes have replaced natural dyes and have become a common practice in textile dyeing due to their bright colours, ease of use, and availability. The decline of natural dyes was due to the rather unscalable production, dull-looking colours compared to artificial dyes, and their sensitivity to light, air, and water.[1] Recently, synthetic dyes have been under increased scrutiny due to their non-sustainability, the use of toxic chemicals, and many health risks.[1] This has led to an increase in the demand for an eco-friendly and healthier alternative to synthetic dyes, hence the resurgence of natural dyes. These ancient natural dyes have been the best way of dyeing textiles and clothing products for millennia, including Batik.[1,2] Indonesian Batik (Figure 1) has been recognized by the United Nations Educational Scientific and Cultural Organization (UNESCO) as an indisputable cultural heritage of Indonesia. Interestingly, exports of Indonesian batik to countries affiliated with the European Union were limited due to the application of artificial dyes, which has been of serious environmental and public health concern.[3]

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Several green batik industries have grown across Indonesia, helping to turn batik into a clean, renewable, and healthy industry.[3]

Natural dyes are used to dye textiles and serve as colourings in food, cosmetics, and pharmaceuticals with several health benefits. The natural dyes such as *Quercus infectoria* and *Quercus infectoria* were reported to exhibit interesting anti-microbial effects, potentially overcoming some of the shortcomings of synthetic dyes.[4] In addition, natural dyes have also been known as a source of antioxidants such as anthocyanins, a well-known phenolic compound with potent antioxidant activity.[4] The uses of natural dyes were also related to their protective capability against ultraviolet (UV) radiation. UV-B radiation can generate free radical species and endanger the functionality of cellular components. Several natural dyes such as fabrics treated with buckthorn leaves, or berries have been reported to protect against ultraviolet radiation, and offer protection from the damaging effect of sun rays.[5]

In the current study, parts of plants, namely; wood of *Caesalpina sappan* L. (Local name: *Secang*), flower of *Clitoria ternatea* L. (Local name: *Telang*), and whole plant of *Mimosa pudica* L. (Local name: *Putri malu*) (Figure 2) commonly used as sources of natural dyes in Indonesian Batik industries were evaluated for their health benefit. *Secang*, *Telang* and *Putri malu* are known to produce red, purple and brown natural dyes, respectively. There are no reports regarding their antibacterial, antioxidant and their UV-screen capability. Therefore, these natural dyes were subjected to an antibacterial assay against common bacteria that causes skin diseases, such as *Staphylococcus aureus*. Their antioxidant capability was determined based on scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. In addition, protective capability against the damaging UV rays from the sun was evaluated. Overall, this investigation provides novel insight into the health benefits of Indonesian batik natural dyes sourced from *Secang*, *Telang* and *Putri malu*.

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Figure 1: The art of traditional batik in Indonesia using natural dyes

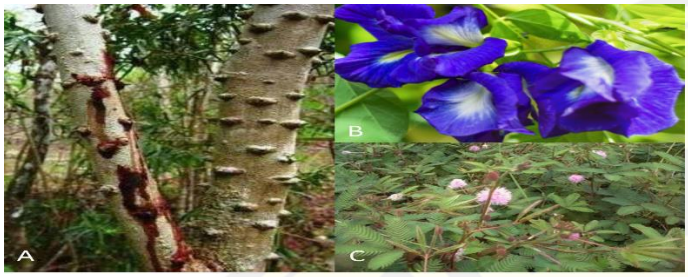

Figure 2: (A) *Secang* (*Caesalpina sappan* L.) tree, (B) *Telang* (*Clitoria ternatea* L.) flower, (C) *Putri malu* (*Mimosa pudica* L.) whole plant

Materials and Methods

Chemicals and regents

NaCl (≥99% analytical grade, Merck, Germany), $BaCl_2$ (≥ 99%, analytical grade, Merck, Germany), Dimethyl sulfoxide (DMSO) (≥99% analytical grade, Merck, Germany), Mueller Hinton Agar (microbiology grade, Merk, Germany), Mueller Hinton Broth (microbiology grade, Merck, Germany), Gentamicin (SanbeFarma Ltd, Indonesia), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) (≥98.5%, analytical grade, Merck, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH((≥ 99%, analytical grade, Merck, Germany), Sterile water (Pyrogen free grade, Ikapharmindo Putramas Ltd).

Equipment

Laminar air flow cabinet (1300 Series A2, Thermo Scientific, USA), Incubator (SIC50L, B-one Shaking Incubator, China), Analytical balance (AX224, Ohaus, USA), Autoclave (ES-315, TOMY, China), Microplate reader (ZN-320, Rayto Life and Analytical Sciences Co., Ltd., China), Gas Chromatography-Mass Spectrometer (Trace 1310 ISQ LT, Thermo Scientific, USA), UV-Vis spectrophotometer (UV-1780, Shimadzu, Japan), Hotplate (MR3001, Heidolph, Germany), Vortexer (Reax Top, Heidolph, Germany), Micropipette (10-100 µL, 100-1000 µL, Eppendorf, Germany).

Plant collection and identification

Dried samples of *Secang* (*Caesalpina sappan* L.), *Telang* (*Clitoria ternatea* L.), and *Putri malu* (*Mimosa pudica* L.) were obtained from a local market at Jember, Jawa Timur-Indonesia (S 8°10'15.752", E 113°41'43.781") in 2023. Samples were transported to the Faculty of Pharmacy, Universitas Jember, and they were authenticated by a taxonomist, Dr. Fuad Bahrul Ulum, Laboratory of Botany, Biology Department, University of Jember, Indonesia. Voucher specimen were deposited at Drug Utilization and Discovery Research Group, Faculty of Pharmacy, Jember University under accession number SC1, TL1 and PM1 for *Secang*, *Telang*, and *Putri malu*, respectively.

Plant samples preparation and dyes extraction

A portion of each sample; 20.91 g of *Secang*, 20.21 g of *Putri malu*, and 20.42 g of *Telang*, were loaded separately in a 1 L pot containing 500 mL of boiling water. The mixtures were left for about 40 minutes in a medium heat until 50 mL of concentrated liquid remained. Supernatants were separately loaded into a 50 mL falcon tube followed by freeze-drying to produce dried dyes with 0.8013 g of *Secang*, 0.2713 g of *Putri malu*, and 2.5968 g of *Telang*.

Determination of UV absorbing capacity

The ultraviolet absorbing capacity of each of the extract solution (100 µg/mL) was determined by the analysis of UV spectra collected from 200 to 400 nm.[6]

Evaluation of antioxidant activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as previously described.[7,8] Crude extracts were prepared in a series of concentrations (400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL) and 100 µL of each concentration was loaded into each well of 96-well plates. DPPH solution (100 µL, 0.2 mM) was added into each well and incubated at room temperature in the dark for 2 h. Methanol was used as a blank, and vitamin C was used as the positive control. The absorbance of the reaction mixture was recorded at 517 nm. Inhibition percentage was calculated using the formula in equation 1.

$$\text{DPPH scavenging effect} = \frac{(A_B - A_A)}{(A_B)} x100\% \quad \text{.....Eq. 1}$$

Where;

A_A is the absorbance of sample or standard, and A_B is the absorbance of DPPH.

Evaluation of antibacterial activity

The antibacterial activity was evaluated according to standard protocol as described by Pamungkas *et al* (2021).[9] Mueller-Hinton agar (7.6 g) was mixed with 200 mL of distilled/sterile water in a 1 L Erlenmeyer flask containing 1 mL of DMSO to aid dissolution. The flask was plugged, and the solution was sterilized in an autoclave at 120ºC for 20 min. The media were transported into a laminar air-flow cabinet and sterilized with Ultraviolet C (UVC) (100 – 280 nm) light for 30 min. A colony of *Staphylococcus aureus* ATCC 6538 was suspended in 0.9% NaCl, and the solution was adjusted until it produced an absorbance value of 0.08 - 0.13 at 625 nm. The antibacterial test was conducted using the disc-diffusion method with a disc diameter of 6 mm. The discs were loaded with positive control (Gentamicin 25 µg), negative control (DMSO 0.5%) or test samples at different concentrations of 1%, 5%, 10%, 15%, and 30%. Antibacterial activity was measured based on the inhibition zone diameter measured in mm. The test was performed in triplicate.

Gas chromatography analysis

Samples were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) following the derivatisation process.[10] Before the derivatization process, samples were stored in a vacuum concentrator at -80°C for 30 minutes, then methoxyamine (40 µL) was added to aliquots of the sample, thereafter, the sample tube was shaken at 37°C for 2 hours. In a separate tube, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was mixed with 20 µL/mL of retention time index standard mixtures, then 70 µL of this solution was mixed with sample aliquots and then shaken for 30 minutes at 37°C. For analysis, 1 µL of derivatized samples were loaded in spitless mode into Trace gold TG-5MS capillary column. Initially, the column temperature was set at 50°C for 2 minutes, then increased to 5°C every minute until it reached 150°C for 10 minutes. Thereafter, it increased by 10°C per minute until it reached 200°C, which remained steady for 5 minutes. Finally, the temperature was increased to 15°C per minute until it reached 320°C, which was held for 5 minutes. During the separation process, the carrier gas (hydrogen) was generated by a hydrogen generator, which has a flow rate of 1 mL per minute. In addition, the injector and transfer line were set at 200°C and 320°C, respectively. Compound annotation was generated from spectral data comparison against the mass spectra library (NIST version 2.2).

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Statistical analysis

Data were presented as Mean ± Standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) using Microsoft Excel 2019 software (Microsoft Corporation). Statistical significant difference was set at *P*-value < 0.05.

Results and Discussion

In the Batik industries, boiling has become a common practice in natural dye extraction. The current study successfully mimicked the traditional protocol in producing dye extracts from *Secang*, *Telang* and *Putri malu* with distinct colours of red, blue, and brown, respectively (Figure 3).

UV radiation is composed of UVC radiation (100 - 280 nm), UVB radiation (280 - 315 nm) and UVA radiation (315 - 400 nm).[11] Despite the short wavelength, UVC radiation has the most dangerous impact on living organisms; it never reaches the earth surface due to the protection of the stratosphere. Meanwhile, the UVB radiation can reach the earth surface due to ozone depletion. UVB radiation is dangerous to cells as direct absorption by the genetic component can cause significant damage and lead to genetic mutation.[8] Compared to UVB and UVC, the less dangerous UVA reaches the earth surface with fewer atmospheric obstacles. In the current investigation on ultraviolet screen or photo absorbent properties of Batik natural dyes, *Secang*, *Telang* and *Putri malu* showed UV absorptivity at wavelength range of 200 to 400 nm where *Secang* showed the highest photo absorbent capability against UVB radiation (Figure 4A). Nowadays, protection from UVB radiation has become a serious issue with the production of several sunscreen products with different Sun Protection Factor (SPF) values depending on the situation. On the other hand, clothes have been part of mankind's adaptation against cellular damage, including the use of dyes with radiation quenching effect.

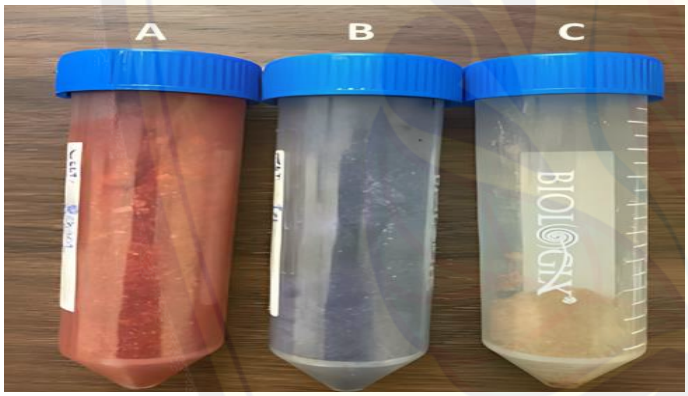

Figure 3: Freeze-dried natural dye of (A) *Secang*, (B) *Telang*, and (C) *Putri malu*.

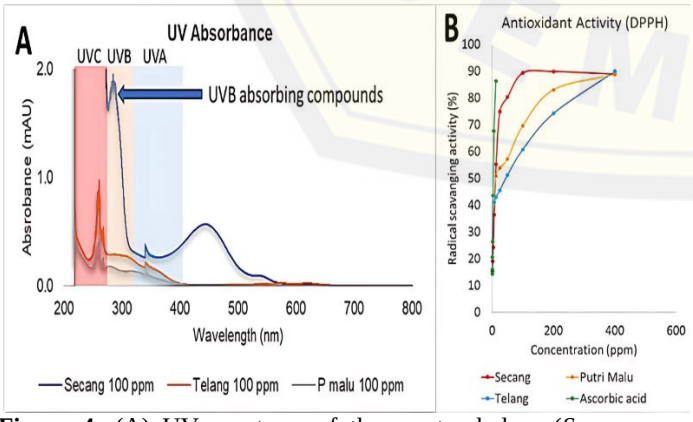

Figure 4: (A) UV spectrum of three natural dyes (*Secang*, *Telang* and *Putri malu*) (B) Antioxidant activity of three natural dyes (*Secang*, *Telang* and *Putri malu*).

Oxidative stress caused by the indirect effect of ultraviolet radiation leads to the formation of reactive oxygen species in the cytoplasm, which can lead to cellular damage.[11] The natural dyes used in Batik have various antioxidant activities. Moreover, the antioxidant activity evaluation of the dyes indicated a significant quenching capability against DPPH radical with IC_{50} values of 10.76, 47.11, and 15.73 µg/mL for *Secang*, *Telang*, and *Putri malu*, respectively, while the positive control (vitamin C) had IC_{50} value of 3.98 µg/mL. Overall, the antioxidant experiment suggested the red colour dye of *Secang* is unique not only for artistic purposes but also for its potential health benefits as a result of its protecting effect from ultraviolet radiation as well as from oxidative stress. In a previous study, several common dyes, including *acacia catechu, Kerria lacca*, *Quercus infectoria*, *Rucia cordofilia*, and *Rumex maritimus* were reported to have antimicrobial effects against several common pathogenic microbes, including *Staphylococcus aureus*, *Escherichia coli*, *Basilicus subtilis*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*.[12,13] Humans are commonly exposed to these pathogens in most environments; and these textile dyes have been shown to exhibit anti-microbial effects, hence would improve the overall health of the user, and improve existing clothing for infants, the elderly, and the vulnerable.[13]

In the current study, three Batik dyes from *Secang*, *Telang* and *Putri malu* were subjected to anti-bacterial activity screening, in which, based on the Clinical & Laboratory Standards Institute (CLSI) method, *Secang* dye was the only plant sample that possessed anti-microbial activity against common skin pathogen *Staphylococcus aureus* (Table 1, Figure 5). The other natural dyes from *Telang* and *Putri malu* had no antibacterial effect, even at the highest concentration of 30% (Figure 5). The phytochemical investigation was conducted using a metabolomic approach based on a gas chromatography experiment in which a derivatization technique was employed, ensuring nonvolatile compounds were conjugated and then compatible for gas chromatographic analysis. The GC chromatograms for the three samples are presented in Figure 6. Further investigation focused on the antimicrobial dye, *Secang*, in which a total of 35 compounds were successfully identified (Table 2, Figure 7).

Table 1: Antibacterial activity of Batik natural dyes against *Staphylococcus aureus*.

Concertation (mg/mL)	**Inhibition zone diameter (mm)**		
	Secang	*Telang*	*Putri Malu*
1	-	-	-
5	9.90 ± 0.17[a]	-	-
10	11.36 ± 0.21[b]	-	-
15	16.09 ± 0.95[c]	-	-
30	19.45 ± 1.05[d]	-	-
Gentamicin (25 µg)	21.26 ± 0.50[e]	-	-

Values represent mean ± standard deviation (SD). Note: (-) not active. Values with different letters are significantly different ($P \leq 0.05$).

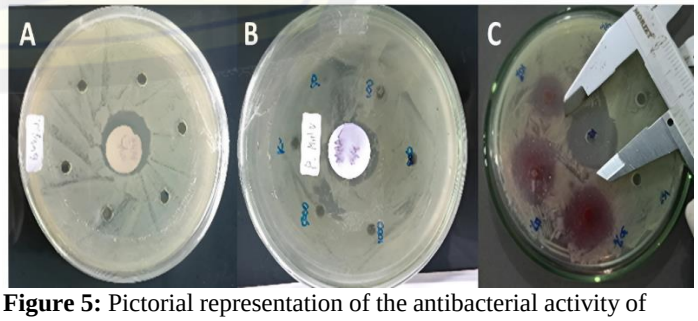

Figure 5: Pictorial representation of the antibacterial activity of the natural dyes of batik. (A) *Telang* dye with no antibacterial effect, (B) *Putri malu* dye with no antibacterial effect, and (C) *Secang* dye showing zone of inhibition.

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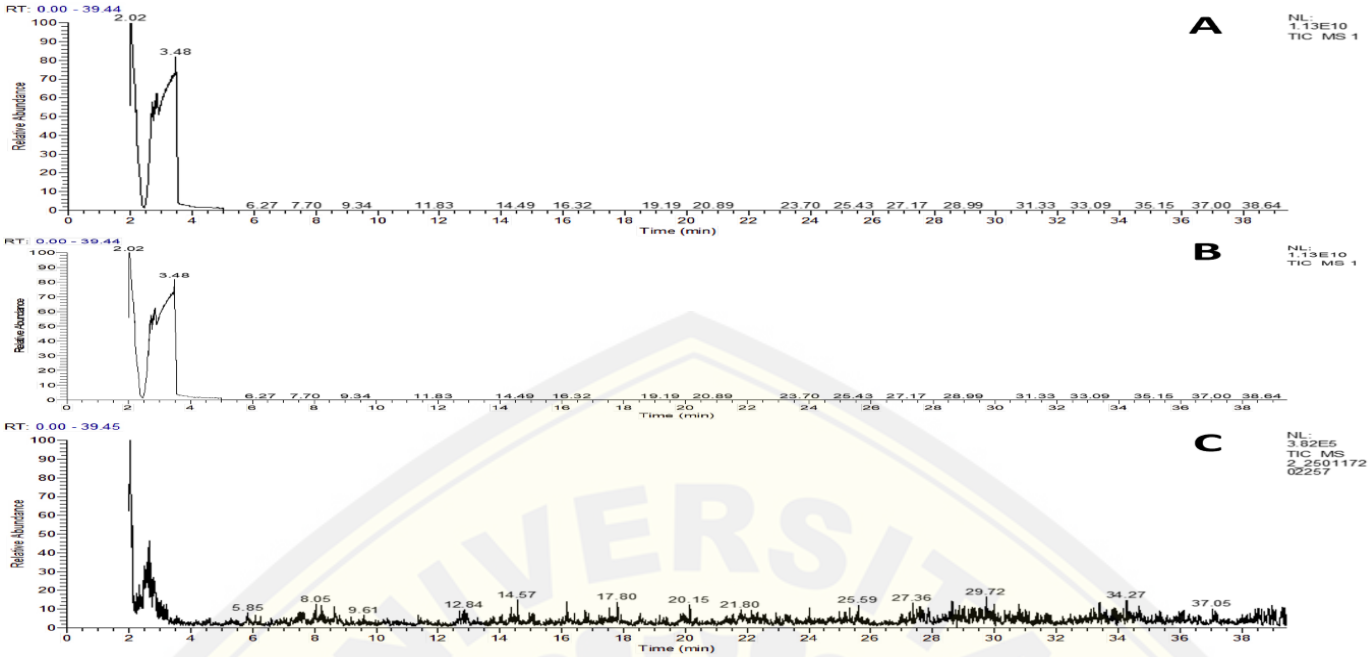

Figure 6: GC chromatogram of natural dyes of batik (A) *Secang*, (B) *Telang* and (C) *Putri malu*

Table 2: Compounds identified from the GC-MS analysis of *Secang* crude extract

No.	Compound Name	RT	RSI	Molecular Formula	Molecular Weight
1	2-[2-(2-hydroxyethoxy)ethoxy]ethanol	2.03	726	$C_6H_{14}O_4$	150
2	Pentaethylene glycol	2.03	661	$C_{10}H_{22}O_6$	238
3	(*R*)-3-Hydroxybutyric acid, methyl ether, methyl ester	2.32	604	$C_6H_{12}O_3$	132
4	1-Allyl(dimethyl)silyloxypropane	2.32	550	$C_8H_{18}OSi$	158
5	2-Dimethyl(prop-2-enyl)silyloxypropane	2.32	560	$C_8H_{18}OSi$	158
6	Octanoic acid, 3-methoxy-, methyl ester	2.32	660	$C_{10}H_{20}O_3$	188
7	3-Methyl-3-buten-1-ol	2.55	581	$C_8H_{18}OSi$	158
8	5-*aza*-bicyclo(2.2.0)hex-2-ene-6-one	2.71	649	C_5H_5NO	95
9	2-Hexyl-1-(2'-pyridinyl)-pyrrolidine	2.83	873	$C_{15}H_{24}N_2$	232
10	(E)-6-(tert-Butyldimethylsiloxy)-2-ethyl-1-(trimethylsilyl)hex-1-ene	2.83	884	$C_{17}H_{38}OSi_2$	314
11	Acetamide, *N*-1,3,2-dioxaborolan-2-yl-2,2,2-trifluoro-*N*-methyl-	3.47	810	$C_5H_7BF_3NO_3$	197
12	*N*-(2-(3,5-Bis[(trimethylsilyl)oxy]phenyl)-2-[(trimethylsilyl)oxy]ethyl)-*N*-(tert-butyl)-2,2,2-trifluoroacetamide	3.76	596	$C_{16}H_{25}NO_7$	343
13	Pyridine	3.88	802	C_5H_5N	79
14	2,4-Pentadienenitrile	3.88	791	C_5H_5N	79
15	Thionicotinamide	4.00	594	$C_6H_6N_2S$	138
16	*N*-Isopropyl-3-(methylsulfanyl)cyclohex-2-enecarboxamide	4.06	709	$C_{11}H_{19}NOS$	213
17	Butanoic acid, 2-amino-, (*S*)-	4.11	981	$C_4H_9NO_2$	103
18	1-dimethylamino-2,3-dimethyl-4-(4-chlorophenyl)-2-butene	4.11	979	$C_{14}H_{20}ClN$	237
19	4-Thiouracil	4.21	688	$C_4H_4N_2OS$	128
20	2-Octanone	4.21	765	$C_8H_{16}O$	128
21	6-Dimethylaminomethyl-3-ethyl-8,9-dimethoxy-3,4-dihydropyrrolo[1,2,3-de]-2H-1,4-benzoxazine methiodide	4.21	794	$C_{18}H_{27}IN_2O_3$	446
22	5-Methyl-5,6-Dihydrouracil	4.31	851	$C_5H_8N_2O_2$	128
23	5-Nitrobarbituric acid	4.31	854	$C_4H_3N_3O_5$	173
24	1-[3-methyl-1-(phenylsulfonyl)-thiopheno[2,3-b]carbazole]-3,5-[bis(3-methyl-2-thiophenyl)]benzene	4.31	625	$C_{37}H_{27}NO_2S_4$	645
25	(*R*)-5,6-Dihydro-4-hydroxy-6-methyl-2H-pyran-2-one	4.41	964	$C_6H_8O_3$	128
26	*N*1-(Formyl)-*N*2-(1-oxobut-2-en-1-yl)hydrazide	4.41	952	$C_5H_8N_2O_2$	128
27	2-(*E*)-(tert-Butoxycarbonylmethylidene)tetrahydrofuran	4.41	939	$C_{10}H_{16}O_3$	184
28	3-(1-Cyclopentenyl)-1-propanol	4.62	871	$C_8H_{14}O$	126
29	*N*-[(2-Methyl-6-heptenyl)-3-oxy]pyridine-2(1*H*)-thione	4.86	929	$C_{13}H_{19}NOS$	237
30	*N*-(1-Cyclohexylpent-4-enyl-1-oxy)pyridine-2(1*H*)-thione	4.86	914	$C_{16}H_{23}NOS$	277
31	*N*-(4-Nitro-2-phenoxyphenyl)methanesulfonamide	4.86	923	$C_{13}H_{12}N_2O_5S$	308
32	4-Phenyl-1-buten-3-yne	4.97	761	$C_{10}H_8$	128
33	Naphthalene	4.97	859	$C_{10}H_8$	128
34	1-Ethanoyl-1,4-dihydronapthalene	4.97	843	$C_{12}H_{12}O$	172
35	Cholesterol	35.15	548	$C_{30}H_{54}O$	458

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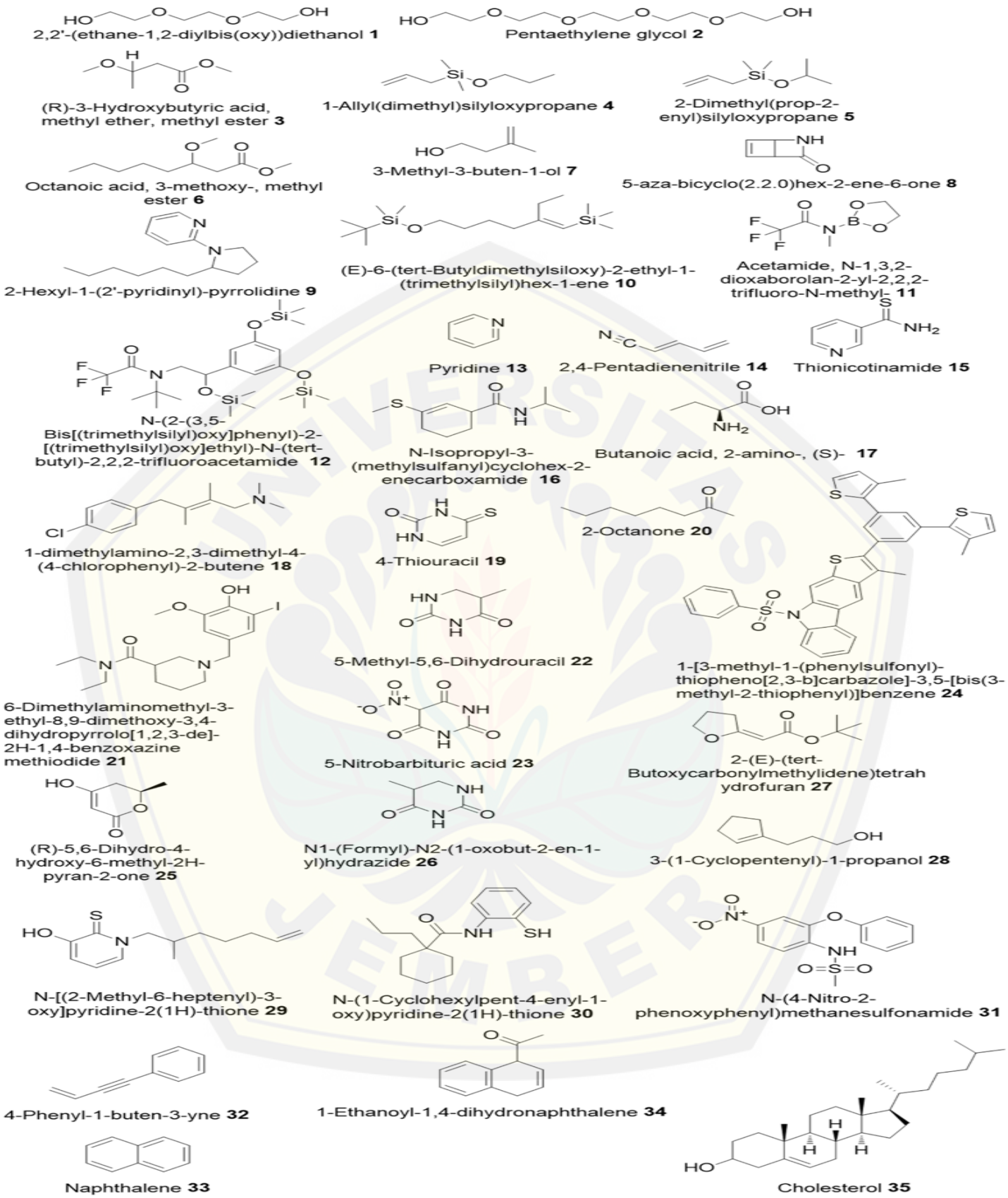

Figure 7: Structures of compounds identified from the GC-MS analysis of *Secang* (*Caesalpina sappan* L.)

Intensive literature search revealed that most of the compounds identified in *Secang* have been reported to possess antibacterial activities. For example, pentaethylene glycol (2) is well-known for its antimicrobial activity.[14] Another study showed that the pyridine derivative (13) has potent antibacterial activity against *Staphylococcus aureus in-vitro*.[15] Investigation on pyridine-coupled pyrazoles indicated a strong antimicrobial activity, with anti-coagulation and anti-biofilm properties.[15] A previous study reported that naphthalene (33) possessed

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an antibacterial effect, while a medicinal chemistry study on a large in-house library of naphthalene-bearing azoles indicated potent antibacterial activity. Several potent derivatives showed significant antibacterial properties against Gram-positive bacteria, such as *Enterococcus faecalis* and *Staphylococcus aureus*. Ethylisobutylmethane functionalized naphthalene showed antibacterial activity with a minimum inhibitor concentration (MIC) of less than 1 mg/mL against *E. faecalis* and *S. aureus*. Further i*n vitro* cytotoxicity studies showed that the active compounds were safe for healthy cells within their MIC ranges, which proved the antibacterial benefits of naphthalene and its derivatives.[16]

Conclusion

Three well-known Batik dyes, *Secang*, *Telang* and *Putri malu* were successfully investigated for their health benefits, including ultraviolet radiation absorbent, antioxidant and antimicrobial activities. Overall, the natural Batik dye *Secang* showed the most promising properties with the ability to absorb UVB radiation (280 - 315 nm), an antioxidant activity with an IC_{50} value of 10.76 µg/mL, and antibacterial activity against *Staphylococcus aureus* with an inhibition zone diameter of 19.45 ± 1.05 mm at a concentration of 30 mg/mL. These results suggest that natural dye in the Batik industries can be used as a prominent alternative to synthetic dyes with health benefits to the wearer. This will preserve the tradition of Batik production, which will improve the economy with environmental consciousness, and empowers communities to embrace their heritage. Nevertheless, investigating other less common Batik dyes is necessary to discover their health benefits.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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