

## Original Research Article

# Determination of Phytochemical Compounds, and Tyrosinase Inhibitory and Antimicrobial Activities of Bioactive Compounds from *Streblus ilicifolius* (S Vidal) Corner

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### Abstract

**Purpose:** To determine the phytochemical content, and tyrosinase inhibitory and antimicrobial activities of the wood from *Streblus ilicifolius* (S. Vidal) Corner

**Methods:** The dried wood of *S. ilicifolius* (8.70 kg) was extracted by maceration to give petroleum ether, ethyl acetate, ethanol and water extracts, respectively. Dopachrome method was used to determine antityrosinase activity. Agar disc diffusion and modified broth microdilution methods were used to determine antimicrobial activity. Chromatographic techniques were used for phytochemical investigation. The structures elucidation of isolated compounds were identified by physical properties and spectroscopic data including UV, IR, NMR and MS data and confirmed by comparison with previously reports.

**Results:** The ethanol extract exhibited tyrosinase inhibition and antimicrobial activity against the Gram-positive bacteria, *Staphylococcus epidermidis* and *S. aureus*. Phytochemical investigation showed five compounds, namely, (E)-2,4-dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde (1), p-hydroxybenzoic acid methyl ester (2), umbelliferone (3), moracin M (4), trans-resveratrol (5). Compound 4 exhibited tyrosinase inhibition with half maximal inhibitory concentration ( $IC_{50}$ ) of 67.69  $\mu\text{g/ml}$ , while compound 1 displayed strong activity against *S. epidermidis*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA) with minimum inhibitory concentration (MIC) of 8, 4 and 8  $\mu\text{g/ml}$ , respectively and minimum bactericidal concentration (MBC) of 32, 16 and 64  $\mu\text{g/ml}$ , respectively.

**Conclusion:** This is the first report of the biological activities and phytochemical composition of *S. ilicifolius* and the results indicate the high potentials of the plant for commercial applications such as in facial whitening and anti-acne cream.

**Keywords:** *Streblus ilicifolius*, Moraceae, Tyrosinase inhibition, Antimicrobial, Anti-acne, Methicillin-resistant *Staphylococcus aureus*

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## INTRODUCTION

Melanin biosynthesis or melanogenesis initiated from L-tyrosine hydroxylated to L-

dihydroxyphenylalanine (L-Dopa), then oxidation of L-Dopa to its corresponding o-dopaquinone, catalyzed by tyrosinase enzyme. o-Dopaquinone can be divided into two different types of reaction

to produce eumelanin and pheomelanin [1,2]. The major function of melanin is to protect against ultraviolet (UV) radiation. However, excessive level of melanin or hyperpigmentation due to a darker or uneven of skin color, age spots, melasma and sites of actinic damage [1].

Tyrosinase inhibitors are becoming increasingly important contain in the treatment products of some dermatological disorders, associate with melanin hyperpigmentation and contain in cosmetic for skin whitening products [1]. Many problems from current whitening cosmetics such as ochronosis, allergic and irritant contact dermatitis, melanocyte toxicity and carcinogenicity [3] cause to find out the new natural whitening agents.

Pathogenic bacteria and dermatophyte fungus are a major cause of human skin disease. Moreover, some microorganisms can be stimulating the melanogenesis [4-7]. Antibiotics are the choice of treatment. However, the use of antibiotics may lead to drug resistance of many bacterial strains. Development of new antimicrobial compounds for resistant organisms is becoming critically important [8].

It is prompt search to find alternative agents from plants that have already been used topically to treat hyperpigmentation in skin of color [1,3,9]. The discovery of tyrosinase inhibition from medicinal plants is alternatives may provide leads for anti-pigmentation compounds and development to whitening and anti-browning agents.

In Thailand, there are many species of the plants in the genus *Streblus*, but only *S. asper* has been reported in traditional uses, and also their pharmacological activities and chemical constituents. However, in this study we have the focus on biological activities of *S. ilicifolius* wood extract. The extract showed the potential of antityrosinase and antimicrobial activities. The aim of this study was to evaluate some biological activities and chemical constitution of *S. ilicifolius*.

## EXPERIMENTAL

### Plant material

140 samples from 79 Thai medicinal plant extracts of Rajjaprabha Dam, Surat Thani Province were screened for antityrosinase activity.

*S. ilicifolius* (S. Vidal) Corner was collected from Rajjaprabha Dam, Surat Thani Province. The plant was identified by Ms. Nannapat Pattharahirantricin, botanist at Plant taxonomy Office of the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand. The voucher specimen number of this plant was SKP 117 19 09 01. It was collected at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

### Extraction and isolation

The dried wood of *S. ilicifolius* (8.7 kg) was chopped, ground and macerated with petroleum ether three times (3 days, each). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 °C. to yield a petroleum ether extract. Then the marc was extracted three times with ethyl acetate and ethanol (3 days, each). It follows an extraction with boiling water. Removal of the solvents gave an ethyl acetate, ethanol and H<sub>2</sub>O extract, respectively. The ethanol extract, showed the potential activities of antityrosinase and antimicrobial was selected for phytochemical investigation. The extract was fractionated by quick column chromatographic technique using a sintered glass-filter column of silica gel 60 (Merck). It was performed a gradient elution using mixtures of chloroform and ethyl acetate (10:0 to 2:8). Finally ethyl acetate was used to wash the column until the eluates were diluted and clear. The result was 13 fractions A-M. Repeated column chromatography of fraction A, gradient elution was performed using mixtures of hexane and ethyl acetate (9:1 to 1:1), given to 15 subfractions. The subfraction A-7 was purified by repeat column chromatography to obtain compound 1. Repeated column chromatography of fraction B, separation was carried out with gradient elution, using mixtures of hexane and ethyl acetate (7:3 to 1:1), to give 8 subfractions. The subfraction B-2 was further fractionated by repeated column chromatography to obtain compound 2 and 3. Repeated column chromatography of fraction E, gradient elution was performed using mixtures of chloroform and methanol (10:0 to 1:1). 12 subfractions were isolated. The subfraction E-8 showed the major spot was further fractionated by repeated column chromatography and crystallized (chloroform-methanol, 9:1) to obtain compound 4 and 5.

### General experimental procedures

The structure of compounds was characterized by NMR spectroscopy and mass spectrometry.

$^1\text{H}$  and  $^{13}\text{C}$  NMR, COSY, HMQC and HMBC spectra were recorded on Varian Unity Inova spectrometer operating at 500 MHz for  $^1\text{H}$ -NMR and 125 MHz for  $^{13}\text{C}$ -NMR and Bruker Avance III ( $^1\text{H}$ -NMR 300 MHz and  $^{13}\text{C}$ -NMR 75 MHz). Mass spectra were measured on Thermo Finnigan MAT 95 XL mass spectrometer. The EI-MS of the compound was determined by GC-MS analysis using an Agilent 7890A GC system interfaced to an Agilent 5975C MSD operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C. A split injection (1  $\mu\text{l}$  injection volume, split ratio, 20:1) at 260 °C injector temperature was utilized. A fused silica capillary column 5 % phenyl 95 % methyl polysiloxane (HP-5MS 30 m x 250  $\mu\text{m}$  x 0.25  $\mu\text{m}$ , Agilent J & W, USA) was used. The temperature program was as follows: 1 min at 60 °C, then to 220 °C at 10 °C/min, then to 300 °C at 4 °C/min, temperature kept at 300 °C for 10 min. The carrier gas was helium 5.6 at a flow rate 1.2 ml/min. Data acquisition was performed with Agilent GC/MSD ChemStation Version E.02.00 for the mass scan range 40-700 u. Column chromatography was carried out using silica gel 60 (particle sized 0.040-0.063 mm). All reaction steps were checked by thin-layer chromatography (TLC). The analyses were performed on pre-coated silica gel 60 F254 plates. Spots on TLC were visualized under UV absorption (254 and 366 nm). For this purpose, the plate was sprayed with anisaldehyde/ $\text{H}_2\text{SO}_4$  reagent followed by heating.

#### Determination of antityrosinase activity

Dopachrome method was used to determine antityrosinase activity, using L-Dopa as the substrate [10]. Dopachrome is one of the intermediate substrate in melanogenesis, this method used the L-Dopa as a substrate. The red color of dopachrome from the oxidation of L-Dopa can be detected by visible light at 492 nm. Antityrosinase activity was performed in 96 well microplate. The samples were dissolved in DMSO to concentration of 200  $\mu\text{g}/\text{ml}$ . The first 20  $\mu\text{L}$  of the samples solution were mixed with phosphate buffer (pH 6.8) and 20  $\mu\text{L}$  of mushroom tyrosinase solution (203.3 U/ml) and incubated at 25 °C for 10 min. Then 20  $\mu\text{L}$  of L-Dopa (0.85 mM) was added. The visible absorption was measured at 492 nm. The solution was incubated at 25 °C for 20 min. After incubation the amount of dopachrome in the reaction was measured at 492 nm again. Tyrosinase inhibition (T) was calculated as in Equation 1.

$$T (\%) = \frac{\{(A - B) - (C - D)\}}{(A - B)} \times 100 \dots\dots (1)$$

where A is the difference of optical density before and after incubation of control, B is the difference of optical density before and after incubation of blank control, C is the difference of optical density before and after incubation of test sample, and D is the difference of optical density before and after incubation of blank sample.

The concentration of the pure compound was measured which caused a 50 % inhibition of tyrosinase activity ( $\text{IC}_{50}$ ). Kojic acid and water extract of *Artocarpus lakoocha* wood were used as positive controls while DMSO was used as negative control.

#### Determination of antimicrobial activity

Microorganisms; *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (TISTR 517), *Propionibacterium acnes* (DMST 14916) and Methicillin-resistant *Staphylococcus aureus* (MRSA 1350II 06) were alternate for gram positive bacteria. *Escherichia coli* ATCC35218 and *Pseudomonas aeruginosa* ATCC10145 were alternate for gram negative bacteria. *Candida albicans* (TISTR 5779) was alternate for yeast and *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* were alternate for fungi. The preliminary screening of antimicrobial activity was used agar disc diffusion method [11]. The pure compounds were determined for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in 96 well microplate by modified broth microdilution method [12,13]. Oxacillin, norfloxacin, amphotericin B and ketoconazole were used as positive controls for gram positive bacteria (except MRSA using vancomycin), gram negative bacteria, yeast and fungi, respectively.

## RESULTS

#### Antityrosinase activity

From 140 samples, *S. ilicifolius* ethanol wood extract showed the highest activity of antityrosinase with  $69.05 \pm 5.00$  %.

#### Antimicrobial activity

The ethanol extract of the wood from *S. ilicifolius* showed the potential antibacterial effects against *S. epidermidis* and *S. aureus* with inhibition zone  $9.25 \pm 0.56$  and  $8.47 \pm 0.31$  mm, respectively (Table 1).

### Extraction of *S. ilicifolius* wood

The dry weight, % yield (base on dried wood) and % tyrosinase inhibition of these crude extracts from the wood of *S. ilicifolius* are shown in Table 2. The ethanol extract was the most interesting fraction because of the potential effects of antityrosinase and antimicrobial activities. So it was selected for isolation of the chemical constituents.

### Structure of isolated compounds

Five pure compounds were isolated from ethanol extract of *S. ilicifolius* (Figure 1). The structures of the isolated compounds, identified by physical properties and spectroscopic data, including UV, IR, NMR and MS data and confirmed by comparison with previously reports are as (E)-2,4-dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde (1) [14], p-hydroxy benzoic acid methyl ester (2) [15], umbelliferone (3) [16], moracin M (4) [17] and trans-resveratrol (5) [18]. Their spectral data are given as follow:

### (E)-2,4-Dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde (Compound 1); C<sub>17</sub>H<sub>22</sub>O<sub>3</sub>

Compound 1 was obtained as colorless needles, soluble in chloroform. The melting point showed at 72.33 °C (Differential scanning calorimeter). The EI mass spectrum of compound 1 exhibited a molecular ion at 274 *m/z*, consistent with a molecular formula of C<sub>17</sub>H<sub>22</sub>O<sub>3</sub>, confirmed by HR-EIMS which showed a molecular ion peak at *m/z* 274.1560 calcd. for C<sub>17</sub>H<sub>22</sub>O<sub>3</sub>, 274.1566. The UV spectrum in chloroform demonstrated absorption maximum in chloroform at λ<sub>max</sub> (absorbance) 228 (0.180), 242 (0.235), 286(0.593) and 396(0.007) nm. The IR spectrum exhibited maximum absorption bands at 3401, 2923, 2851 and 1622 cm<sup>-1</sup>. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 1 are showed as:

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): 1.57 (3H, s, H-9'), 1.65 (3H, s, H-10'), 1.80 (3H, s, H-4'), 2.06 (4H, m, H-5', H-6'), 3.43 (2H, d, *J*=7.3 Hz, H-1'), 5.02 (1H, m, H-7'), 5.24 (1H, tq, *J*=7.3, 1.1 Hz, H-2'), 6.28 (1H, s, 4-OH), 6.46 (1H, d, *J*=8.4 Hz, H-5), 7.28 (1H, d, *J*=8.4 Hz, H-6), 9.66 (1H, s, 1-COH), 11.75 (1H, s, 2-OH);

**Table 1:** Antimicrobial activity of *S. ilicifolius* solvent extract

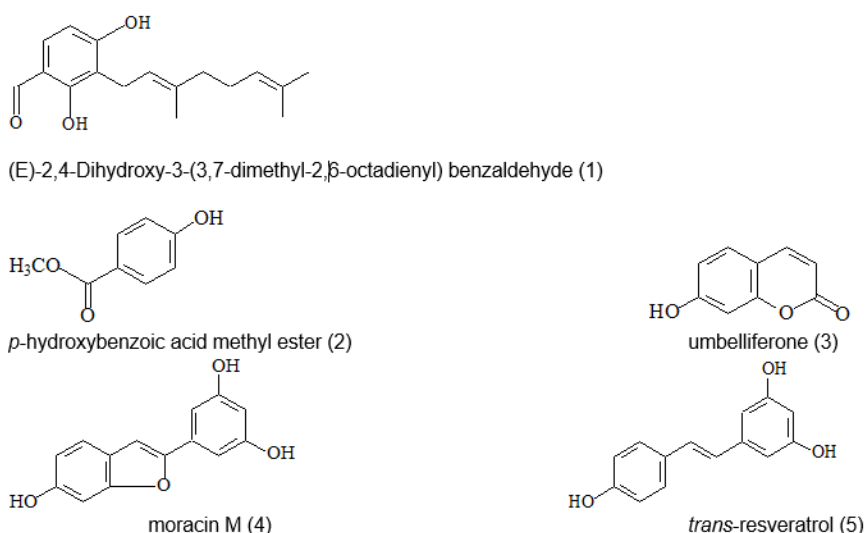
Microorganism	Diameter <sup>a</sup> of inhibition zone (mm)				Positive control
	<i>Pet. ether</i>	<i>EtOAc</i>	<i>EtOH</i>	<i>Water</i>	
<b>Gram-positive bacteria</b>					
<i>S. epidermidis</i>	-	-	9.25±0.56	-	28.01±0.55 <sup>b</sup>
<i>S. aureus</i>	-	-	8.47±0.31	-	18.73±0.40 <sup>b</sup>
<i>P. acnes</i>	-	-	-	-	37.83±2.84 <sup>b</sup>
<b>Gram-negative bacteria</b>					
<i>E. coli</i>	-	-	-	-	28.42±3.42 <sup>c</sup>
<i>P. aeruginosa</i>	-	-	-	-	29.75±0.21 <sup>c</sup>
<b>Yeast</b>					
<i>C. albicans</i>	-	-	-	-	15.77±1.13 <sup>d</sup>
<b>Fungi</b>					
<i>M. gypseum</i>	-	-	-	-	20.97±1.25 <sup>e</sup>
<i>T. rubrum</i>	-	-	-	-	38.50±2.12 <sup>e</sup>
<i>T. mentagrophyts</i>	-	-	-	-	31.01±1.43 <sup>e</sup>

- = no inhibition zone, at concentration of extracts = 2 mg/disc; <sup>a</sup> = includes diameter of disc (6 mm); <sup>b</sup> = oxacillin, <sup>c</sup> = norfloxacin, <sup>d</sup> = amphotericin B, <sup>e</sup> = ketoconazole; *Pet. ether* = petroleum ether extract, *EtOAc* = ethyl acetate extract, *EtOH* = ethanol extract, *Water* = water extract

**Table 2:** Yield and tyrosinase inhibitory activity of *S. ilicifolius* crude extract (at final concentration 20 µg/ml)

Crude extract	Dry weight (g)	Yield (%)	Tyrosinase inhibition (%)
Petroleum ether	9.75	0.11	2.45± 5.90
Ethyl acetate	23.21	0.27	24.38± 1.04
Ethanol	109.78	1.26	75.52± 5.42
H <sub>2</sub> O	103.96	1.19	3.17± 5.24
Kojic acid <sup>P</sup>	-	-	80.86 ± 2.62
<i>Artocarpus lakoocha</i> <sup>P</sup>	-	-	87.62± 1.52

<sup>P</sup> = positive control



**Figure 1:** Pure compounds isolated from ethanol extract of *Streblus ilicifolius* wood

**<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):** 16.23 (C-16'), 17.69 (C-9'), 21.33 (C-1'), 25.65 (C-10'), 26.26 (C-6'), 39.67 (C-5'), 109.04 (C-5), 113.50 (C-3), 115.02 (C-1), 120.55 (C-2'), 123.60 (C-7'), 132.16 (C-8'), 133.50 (C-6), 140.06 (C-3'), 161.65 (C-4), 162.70 (C-2), 194.63 (1-COH).

***p*-Hydroxybenzoic acid methyl ester (Compound 2); C<sub>9</sub>H<sub>8</sub>O<sub>3</sub>**

The compound 2 was obtained as a colorless needles, soluble in methanol and dimethyl sulfoxide. The melting point showed at 118 °C. The % abundance GC-MS of compound 2 exhibited a molecular ion at 152 *m/z*, consistent with a molecular formula of C<sub>9</sub>H<sub>8</sub>O<sub>3</sub>. The UV spectrum in methanol demonstrated absorption maximum in methanol at λ<sub>max</sub> (absorbance) 255 (0.837) and 314 (0.121) nm. The IR spectrum exhibited maximum absorption bands at 3355, 1681, 1609, 1585 and 1513 cm<sup>-1</sup>. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 2 are showed as:

**<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>):** 3.78 (3H, s, 7-OCH<sub>3</sub>), 6.84 (2H, d, *J*=8.70 Hz, H-2, H-6), 7.80 (2H, d, *J*=8.68 Hz H-3, H-5), 10.34 (1H, brs, 4-OH);

**<sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>):** 52.06 (7-OCH<sub>3</sub>), 115.78 (C-2, C-6), 120.68 (C-1), 131.86 (C-3, C-5), 162.39 (C-4), 166.50 (C-7).

**Umbelliferone (Compound 3); C<sub>9</sub>H<sub>6</sub>O<sub>3</sub>**

The compound 3 was obtained as a yellowish crystalline, soluble in methanol and dimethyl sulfoxide. The melting point showed at 230 °C. It gave a molecular ion at 162 *m/z* in the % abundance GC-MS, suggesting a tentative molecular formula of C<sub>9</sub>H<sub>6</sub>O<sub>3</sub>. The UV spectrum

in methanol displayed absorptions maximum in methanol at λ<sub>max</sub> 216, 258 and 332 nm. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 3 are showed as:

**<sup>1</sup>H-NMR(300 MHz, DMSO-*d*<sub>6</sub>):** 6.19 (1H, d, *J* = 9.45 Hz, H-3), 6.71 (1H, d, *J*=2.28Hz, H-8), 6.78 (1H, dd, *J*=8.49, 2.31 Hz, H-6), 7.52 (1H, d, *J*=8.49 Hz, H-5), 7.93 (1H, d, *J*=9.48 Hz, H-4);

**<sup>13</sup>C-NMR(75 MHz, DMSO-*d*<sub>6</sub>):** 102.61 (C-8), 111.66 (C-4a), 111.77 (C-3), 113.62 (C-6), 130.16 (C-5), 145.01 (C-4), 155.97 (C-8a), 160.91 (C-7), 161.89 (C-2).

**Moracin M (Compound 4); C<sub>14</sub>H<sub>10</sub>O<sub>4</sub>**

The compound 4 was obtained as a cream needles, soluble in methanol and dimethyl sulfoxide. The melting point showed at 270 °C. It gave a molecular ion at 242 *m/z* in the EI mass spectrum, corresponding to C<sub>14</sub>H<sub>10</sub>O<sub>4</sub>. The UV spectrum in methanol showed absorptions at λ<sub>max</sub> (absorbance) 216 (0.863), 315 (0.938) and 328 (0.796) nm. The IR spectrum showed the absorption bands at 3521, 3328, 1613, 1436, 1292 and 1141 cm<sup>-1</sup>. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 4 are showed as:

**<sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>):** 6.20 (1H, t, *J*=2.05 Hz, H-4'), 6.67 (2H, d, *J*=2.05Hz, H-2', H-6'), 6.72 (1H, dd, *J*=8.46, 2.06 Hz, H-5), 6.91 (1H, ddd, *J*=2.06, 0.91 Hz, H-7), 7.05 (1H, d, *J*=0.91 Hz, H-3), 7.37 (1H, d, *J*=8.25 Hz, H-4), 9.39 (2H, brs, 3'-OH, 5'-OH), 9.95 (1H, brs, 6-OH);

**<sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>):** 97.62 (C-7), 101.69 (C-3), 102.47 (C-2', C-6'), 102.81 (C-4'), 112.61 (C-5), 120.94 (C-3a), 121.26 (C-4),

**Table 3:** Tyrosinase inhibitory activity (IC<sub>50</sub>) of pure compounds isolated from *S. ilicifolius*

Compound	IC <sub>50</sub> (µg/ml)
(E)-2,4-Dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde (1)	> 200
<i>p</i> -Hydroxybenzoic acid methyl ester (2)	> 200
Umbelliferone (3)	NT
Moracin M (4)	67.69
<i>Trans</i> -resveratrol (5)	NT
Kojic acid <sup>P</sup>	38.67
<i>Artocarpus lakoocha</i> <sup>WP</sup>	8.73

<sup>W</sup> = water wood extract; <sup>P</sup> = positive control; NT = not tested

**Table 4:** MIC and MBC of isolated compounds against *S. epidermidis*, *S. aureus* and MRSA

Compound	<i>S. epidermidis</i>		<i>S. aureus</i>		MRSA	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	8	32	4	16	8	64
2	256	>256	>256	>256	>256	>256
3	NT	NT	NT	NT	NT	NT
4	64	>256	128	>256	64	>256
5	NT	NT	NT	NT	NT	NT
Oxacillin <sup>P</sup>	0.5	-	0.25	-	-	-
Vancomycin <sup>P</sup>	-	-	-	-	0.5	-

NT = not tested; <sup>P</sup> = positive control; Oxacillin for *S. epidermidis* and *S. aureus*, Vancomycin for MRSA

131.82 (C-1'), 154.13 (C-2), 155.42 (C-7a), 155.88 (C-6), 158.94 (C-3', C-5').

#### *trans*-Resveratrol (Compound 5); C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>

The compound 5 was obtained as a brownish crystalline, soluble in methanol and dimethyl sulfoxide. The melting point showed at 265 °C. It gave a molecular ion at 228 *m/z* in the % abundance GC-MS, suggesting a tentative molecular formula of C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>. The UV spectrum in methanol, showed absorptions at λ<sub>max</sub> (absorbance) 207 (0.647), 315 (0.531) and 328 (0.450) nm. The IR spectrum exhibited maximum absorption bands at 3234, 1618, 1578, 1508 and 1485 cm<sup>-1</sup>. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound 5 are showed as:

<sup>1</sup>H-NMR(300 MHz, DMSO-*d*<sub>6</sub>): 6.10 (1H, brs, *J*=2.10 Hz, H-4), 6.37 (2H, d, *J*=2.13 Hz, H-2, H-6), 6.74 (2H, d, *J*=8.58 Hz, H-3', H-5'), 6.80 (1H, d, *J* = 16.22 Hz, H-α), 6.93 (1H, d, *J*= 16.35 Hz, H- α'), 7.38 (2H, d, *J*=8.55 Hz, H-2', H-6'), 9.20 (2H, brs, 3-OH, 5-OH), 9.56 (1H, brs, 4'-OH);

<sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>): 102.19 (C-4), 104.72 (C-2, C-6), 115.94 (C-3', C-5'), 126.07 (C-α), 128.29 (C-2', C-6', C-α'), 128.49 (C-1'), 139.69 (C-1), 157.65 (C-4'), 158.93 (C-3, C-5).

GC-MS (EI): *m/z* (%) = 228.1[M<sup>+</sup>] (100), 211.1 (10), 181.1 (17), 157.1 (12), 115.0 (9), 44.0(12).

#### Antityrosinase activity of isolated compounds

Determination of tyrosinase inhibitory activity by dopachrome method of isolated compounds was showed in Table 3.

#### Antimicrobial activity of isolated compounds

From the screening of antimicrobial activity, *S. ilicifolius* ethanol extract displayed an inhibition zone against *S. aureus* and *S. epidermidis*. MIC and MBC of isolated compounds were determined with *S. epidermidis*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA). The MIC and MBC values of isolated compounds are showed in Table 4.

#### DISCUSSION

Five pure compounds were isolated from *S. ilicifolius* for the first time although all of them are known compounds. Compound 1 and 2 were inactive with antityrosinase activity. While, compound 4 was found to exhibit a high antityrosinase activity. The hydroxyl group at the 6 position of moracin M has been reported that it might mediate the inhibitory activity [19]. Moracin M is 2-arylbenzofuran, the hydroxyl group at the 6 position might mediate the inhibitory activity, compared with two 2-arylbenzofurans; moracin N and moracin O

(Figure 2). Moracin N and moracin O belong to isoprenyl-substituted 2-arylbenzofuran which showed the activity against tyrosinase with  $IC_{50}$  30.52  $\mu$ M and 93.58  $\mu$ M, respectively. In moracin O, the isoprenyl group forms a five-membered ring with the hydroxyl group at the 6 position, whereas in moracin N, the isoprenyl group remains intact which might contribute to its higher tyrosinase inhibitory activity than moracin O [19].

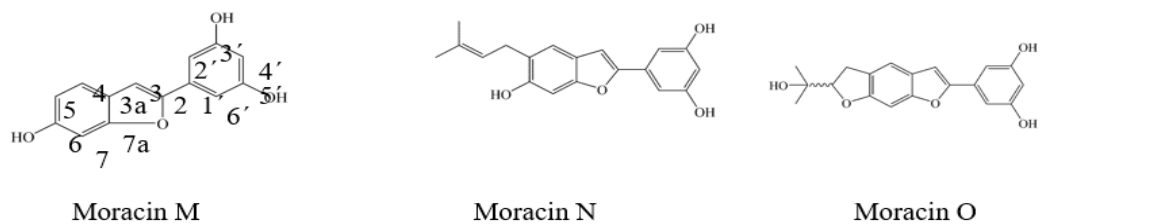
The activity of tyrosinase inhibitors are classified into four types, including competitive inhibitors, uncompetitive inhibitors, mixed type (competitive/uncompetitive) inhibitors, and non-competitive inhibitors [26]. Resveratrol is a polyphenolic phytoalexin that belongs to the stilbenes, which have demonstrated potent tyrosinase inhibitory activity [27]. This substance acts as a "suicide substrate" for tyrosinase [28]. While, umbelliferone is a coumarin analog with hydroxyl group at C7 could be competitive potent tyrosinase inhibitory activity like the other coumarin, esculetin [29]. Moreover moracin M, the arylbenzofuran showed competitive potent tyrosinase inhibitory activity [30].

(E)-2,4-Dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde (1) showed strong inhibitory effect against *S. epidermidis*, *S. aureus* and MRSA. Friedman *et al* [25]. reported that 2, 3-dihydroxybenzaldehyde was highly active against four bacteria (*Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*) because hydroxyl group at the position

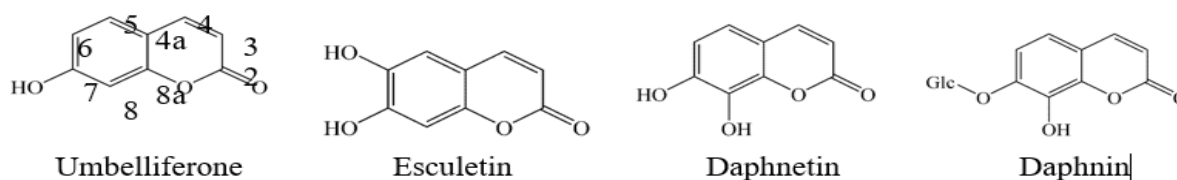
2 and 3 enhance the antimicrobial activities of benzaldehyde. Then, hydroxyl group at the position 2 and 3 of 2, 3-dihydroxy-4-geranyl benzaldehyde might mediate the inhibitory activity.

Moracin M (4) is the arylbenzofuran, showed weak inhibitory effect against, *S. epidermidis*, *S. aureus* and MRSA. Mazimba *et al* [30] reported moracin M against *S. aureus* (MIC 62.5  $\mu$ g/mL), *B. subtilis* (MIC 31.25  $\mu$ g/mL), *M. flavus* (MIC 125  $\mu$ g/mL), *S. faecalis* (MIC 62.5  $\mu$ g/mL), *S. abony* (MIC 62.5  $\mu$ g/mL) and *P. aeruginosa* (MIC 125  $\mu$ g/mL). Moreover, Kuetu *et al* [32] reported that all tested microbial species (Gram-positive, Gram-negative bacteria and fungi) were inhibited by non-prenylated arylbenzofurans; moracin M and moracin Q (Figure 4) weaker than prenylated arylbenzofurans; moracin T and moracin C (Figure 4) because prenyl increases the antimicrobial activity of arylbenzofurans.

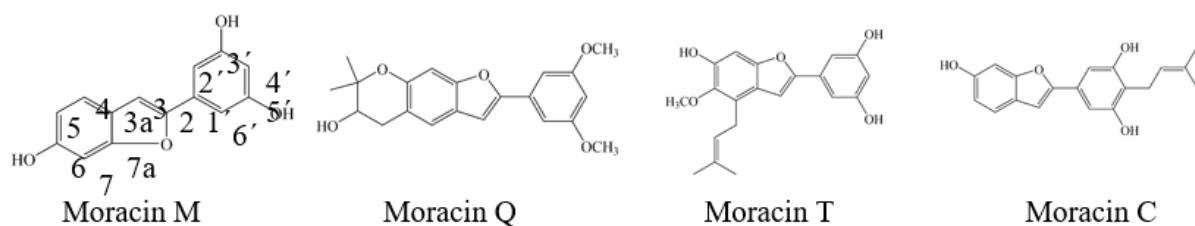
p-hydroxybenzoic acid methyl ester (2) showed very low MIC against *S. epidermidis*. It is one of a homologous series of parabens used to exert antimicrobial affect especially, useful against molds and yeasts. It againsts *A. oryzae* (MIC 600  $\mu$ g/mL), *T. lignorum* (MIC 250  $\mu$ g/mL), *S. lutea* (MIC 4000  $\mu$ g/mL), *E. cloacae* (MIC 1000  $\mu$ g/mL), *P. vulgaris* (MIC 2000  $\mu$ g/mL) (Kibbe, 2000), *E. coli* (wild type) (MIC 1400  $\mu$ g/mL), *E. coli* (envelope mutant) (MIC 1000  $\mu$ g/mL), *P. aeruginosa* (wild-type) (MIC 1800  $\mu$ g/mL) and *P. aeruginosa* (envelope mutant) (MIC 1000  $\mu$ g/mL) [33].



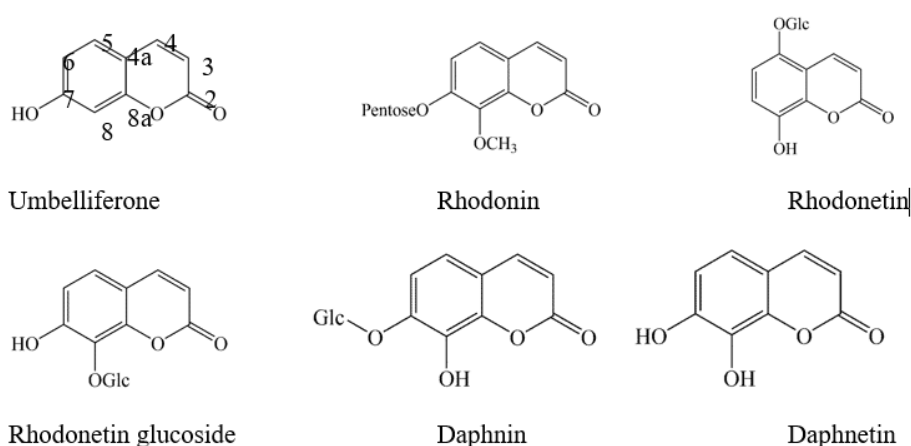
**Figure 2:** Structure of moracin M, moracin N and moracin O



**Figure 3:** Structure of umbelliferone, esculetin, daphnetin and daphnin



**Figure 4:** Structure of moracin M, Q, T and C



**Figure 5:** The structure of umbelliferone, rhodonetin, rhodonetin glucoside, rhodonin, daphnin and daphnetin

Some of the isolated compounds; umbelliferone (3) and trans-resveratrol (5) did not test because they have trace amount. However, they have been reported of anti-microbial activity.

Resveratrol showed activity against Gram-positive bacteria higher than Gram-negative bacteria. It had the highest activity against *Bacillus cereus* followed by *Staphylococcus aureus*, MRSA and *Enterococcus faecalis* [34]. Moreover, Kukric and Topalic-Trivunovic [35] reported that trans-resveratrol showed antimicrobial activity against test organisms (*E. coli*, *B. subtilis*, *Straphylococcus* sp. and *S. lutea*) higher than cis-resveratrol. In addition, trans-resveratrol displayed potent antifungal activity against human pathogenic fungi (*C. albicans*, *S. cerevisiae* and *T. beigellii* with MIC 10, 10-20 and 10 µg/mL, respectively [36].

Umbelliferone (Figure 5), which has a free hydroxyl at the position 7, did not possess good antibacterial activity while, daphnetin which has free hydroxyl at the position 7 and 8, is the most active compound out of all the coumarins tested. Moreover, daphnin and rhodonetin which has free hydroxyl at the position 8, showed better activity than rhodonetin glucoside which the glucoside at the position 8 and rhodonin which methoxyl at the position 8. From the data concluded that coumarins with free hydroxyl at the position 8 possess better antibacterial activity [37].

However, these results will be useful for further study for pharmaceutical cosmetics or application for skin treatment of hyperpigmentation and infectious diseases such as the formulations of cream, serum, mask and so on.

## CONCLUSION

Five pure compounds were isolated from the wood of *S. ilicifolius*. This is the first report on the biological activities and phytochemical profile of *S. ilicifolius*. Also, (E)-2,4-Dihydroxy-3-(3,7-dimethyl-2,6-octadienyl) benzaldehyde from natural products, which has been investigated here for the first time, shows good antimicrobial activity. Moracin M has antityrosinase activity. However, further studies ascertain the actual therapeutic potentials of the compounds in the management of infectious diseases and hyperpigmentation.

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