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Short Communication

Phytochemicals and tyrosinase inhibitory activity from *Piper caninum* and *Piper magnibaccum*

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ABSTRACT

Backgroud: *Piper* species are aromatic plants used as spices in the kitchen, but their secondary metabolites have also shown biological effects on human health. In traditional medicine, *Piper* species have been used worldwide to treat several diseases such as urological problems, skin, liver and stomach ailments, for wound healing, and as antipyretic and anti-inflammatory agents. In the present study, we attempted to isolate the phytochemicals from *Piper caninum* and *Piper magnibaccum* and evaluate their tyrosinase inhibitory activity.

Methods: Phytochemical constituents of the extract were investigated using various chromatographic and spectroscopic methods. The structures of the isolated phytochemicals were established by analysis of their spectroscopic data, as compared to that of reported data. Tyrosinase inhibitory activity was also tested on the extracts and selected compounds using mushroom tyrosinase as the enzyme.

Results: Fractionation and purification of the extracts of *Piper caninum* and *Piper magnibaccum* afforded nine known compounds which are cepharanone A (**1**), cepharadione A (**2**), aristolactam AII (**3**), 5,7-dimethoxyflavone (**4**), 24-methylenecycloartan-3-one (**5**), β-sitosterol (**6**), piperumbellactam A (**7**), 24S-ethylcholesta-5,22,25-trien-3β-ol (**8**) and stigmast-3,6-dione (**9**). Ethyl acetate extracts from leaves of *P. magnibaccum* gave the highest inhibition value at 48.35%, while the tested compounds displayed weak tyrosinase activity compared to the positive control, kojic acid.

Conclusion: These phytochemical results suggest that the extracts could assist as a potential source of bioactive compounds. Further research is needed in which the extract could possibly be exploited for pharmaceutical use.

Keywords: Phytochemical, Piperaceae, *Piper maingayi*, *Piper magnibaccum*, tyrosinase

Introduction

The Piperaceae family is assigned in the order of Piperales and widely distributed in the tropics and subtropic regions. The genus *Piper* is the most representative of the family Piperaceae has

more than 1000 species distributed worldwide. A total of 1200 species of *Piper* distributed in the pantropical and neotropical regions of the world, while over 400 species were documented in Malaysia region.[1] The uses of *Piper* species from Peninsular Malaysia were documented which obviously included the cultivated *P. nigrum*, the primary sources of spices worldwide. Previous studies reported the phytochemical investigations from *Piper* species have led to the isolation of a large number of physiologically active compounds such as alkaloids/amides, lignans/neolignans, flavonoids, triterpenes, steroids, kawapyrones and piperolides [2-4] with numerous bioactivities, such as antioxidant, antimicrobial, antifungal, antityrosinase, anticholinesterase, antituberculosis, antiplasmodial, anti-inflammatory, antileishmanial and insecticidal activities.[5-7]

Piper caninum Blume locally known as "*cabai hutan*"or"*lada hantu*", can be found throughout the tropic mostly in South East Asia.[8] Previously, phytochemical studies have shown the presence of stilbene, flavonoids, phenolic acid amides, alkaloids and bornyl hydroxycinnamic esters.[9-12] *Piper magnibaccum* C.DC. is identified as a woody climber, glabrous with a fleshy stem. The nodes are with adventitious roots and fleshy, coriaceous, elliptic or elliptic-ovate, asymmetric leaves.[13] Previously, we have reported the phytochemicals and their antibacterial and anti-inflammatory activities.[14-15]

In this article, we aim to report detailed phytochemical study and tyrosinase inhibitory activity of *Piper caninum* Blume and *Piper magnibaccum* C.DC. collected from Pahang, Malaysia.

Materials and Methods

Plant materials

P. caninum (SK2331/14) and *P. magnibaccum* (SK2330/14) were collected from Hutan Simpan Fraser, Fraser Hill, Pahang in Jan 2014, and identified by Shamsul Khamis. The voucher specimens were deposited at the Herbarium of IBS, UPM.

General experimental procedures

Solvents systems used in the chromatographic method were; petroleum ether (PE), *n*-hexane, diethyl ether (Et_2O), ethyl acetate (EtOAc), chloroform ($CHCl_3$), dichloromethane (DCM) and methanol (MeOH). Petroleum ether (60-80°C) was distilled before used. Cold extraction technique was applied to extract the phytochemicals from the dried sample. Vacuum liquid chromatography (VLC) was performed on Merck silica gel 60 (230-400 mesh) while column chromatography (CC) on Merck silica gel 60 (70-230 mesh) and Sigma Aldrich MCI-Gel CHP20P as a stationary phase. Thin layer chromatography (TLC) analysis was performed on

Merck pre-coated silica (SiO_2) gel F254 plates with 0.2 mm thickness to detect and monitor compounds present in the crude samples or fractions. The spots were visualized under UV light at 254 and 365 nm, included with spraying reagent vanillin-sulphuric acid in methanol followed by heating. Melting points were measured by using melting point apparatus equipped with a microscope, Leica Gallen III and were uncorrected. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 Spectrophotometer. Chemical shifts were reported in ppm and $CDCl_3$ as the solvent. Residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ATR and 1600 spectrophotometer series as KBr disc or thin film of NaCl discs. High-resolution mass spectral data were recorded on Bruker Mass Spectrometry, obtained from the National University of Singapore (NUS).

Extraction and isolation

The dried aerials (500 g) of *P. caninum* were soaked successively with *n*-hexane to yield *n*-hexane extract, PCAH (8.12 g). The residues dry samples were then soaked with MeOH:H_2O in 9:1 to yield the PCAM:H_2O extract. The extract PCAM:H_2O was further undergoing liquid fractionation using two solvents, *n*-hexane (PCAH) and dichloromethane (PCAD). The PCAH was subjected to VLC (70.0 g silica, column size 6.0 cm x 4.0 cm) and afforded two polled fraction F1-2. Fraction F2 was subjected to CC (Hexane:DCM, 80:20) to afford β-sitosterol (**6**). Fraction F1 was subjected to CC (Hexane:DCM, 70:30) purification to yield 24-methylenecycloartan-3-one (**5**) and 5,7-dimethoxyflavone (**4**). PCAD-D extract was purified by CC (Hexane:DCM, 60:40) to give three compounds from fractions F 25-35, cepharanone A (**1**); F 77-85, cepharadione A (**2**) and F 93-98, aristolactam AII (**3**).

The dried leaves (500 g) and stems (1.5 kg) of *P. magnibaccum* were sequentially subjected to cold extraction with *n*-hexane (5.0 L), EtOAc (5.0 L) and MeOH (5.0 L) successively. The *n*-hexane extract, PMGLH (5.34 g), EtOAc extract, PMGLE (12.89 g) and MeOH extract, PMGLM (8.10 g) of leaves were fractionated using VLC (70.0 g silica, column size 6.0 cm x 4.0 cm). PMGLH and PMGLE were fractionated and eluted with *n*-hexane, $CHCl_3$, EtOAc and MeOH by increasing polarity to give 19 and 29 fractions, respectively. PMGLH fractions were pooled into three fractions, F1-3. Whereas PMGLE were pooled into three fractions, F1-4. PMGLM was directly subjected to CC (Hexane:DCM, 75:25) to afford two combined fractions which were F1-2. The fractions were combined based on TLC profiles. Fraction F1 was fractionated by CC (DCM:MeOH, 95:5) to afford 24*S*-ethylcholesta-5,22,25-trien-3β-ol (**8**), while fraction F2 which was purified by CC (DCM:MeOH, 90:10) has afforded β-sitosterol (**6**). Fraction F1-8 from the PMGLE extract was subjected to VLC fractionation in

$CHCl_3$:EtOAc to afford cepharadione A (**2**) by repetitive CC, and fraction F18-29 was subjected to CC to afford piperumbellactam A (**7**). The stem extracts of *P. magnibaccum* (PMGSH) was purified by CC (DCM:MeOH, 85:15) to afford stigmast-3,6-diene (**9**). The residues dry samples from the successive PMGSH were soaked with MeOH: H_2O (9:1) to afford PMGSM-H_2O extract which was further undergoing liquid-liquid extraction using two solvents *n*-hexane and DCM to yield PMGSM-H and PMGSM-D extracts respectively. The PMGSM-D extract was directly subjected to CC (DCM:MeOH, 80:20) to afford the combined fraction F25-35. Fraction F77-85 underwent CC (DCM:MeOH, 75:15) to afford cepharadione A (**2**) while fraction F93-98 gave piperumbellactam A (**7**).

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was assayed by using the modified dopachrome method with slight modifications with the concentration of sample used was 1000 µg/mL diluted in 20 mM phosphate buffer solution (PBS) with pH 6.8. L-DOPA was used as the substrate and mushroom tyrosinase as the enzyme.[16-17] The assays were conducted in 96-well microtiter plate, and Epoch Biotek reader was used to measure the absorbance at 475 nm. Mushroom tyrosinase enzyme (MTE) was prepared from the stock solution to 480 units/ mL in PBS. For each concentration of the sample solution, four wells designated A, B, C and D each contained a reaction mixture (180 µL) as follows: (A) 20 µL of MTE; (B) 140 µL of PBS, and 20 µL of methanol; (C) 20 µL of MTE, 140 µL of 20 mM PBS and 20 µL of sample solution; (C) 160 µL of PBS and 20 µL of sample solution. Each well was mixed and incubated at 25ºC for 10 min. Then, 20 µL of 0.85 mM L-DOPA in PBS was added. After incubation at 25ºC for 20 min, the amount of dopachrome in each reaction mixture was measured as the difference of the optical density before and after incubation. The percent inhibition of activity was calculated using the equation; I% = 100 [(A-B) - (C-D)] / (A-B); where; A was the difference of optical density before and after incubation without test sample; B was the difference of optical density before and after incubation without test sample and enzyme; C was the difference of optical density before and after incubation with test sample; D was the difference of optical density before and after incubation with the test sample but without enzyme. Kojic acid was used as a positive standard. The test was carried out in triplicate and reported as mean ± SD of triplicate.

Statistical analysis

Data obtained from a biological activity are expressed as mean values. Statistical analyses were carried out by employing one way ANOVA (p>0.05). A statistical package (SPSS version 11.0) was used for the data analysis.

Results and discussion

The medicinal values possessed by the plant of *Piper* genus have led to phytochemical studies by many researchers. It has been reported that hundreds of secondary metabolites have been successfully isolated. In this study, phytochemical investigation on the leaves of two *Piper* species has been investigated. Six compounds have been isolated from *P. caninum* which are cepharanone A (**1**), cepharadione A (**2**), aristolactam AII (**3**), 5,7-dimethoxyflavone (**4**), 24-methylenecycloartan-3-one (**5**), and β-sitosterol (**6**). In addition, cepharadione A (**2**), β-sitosterol (**6**), piperumbellactam A (**7**), 24*S*-ethylcholesta-5,22,25-trien-3β-ol (**8**) and stigmast-3,6-dione (**9**) have been isolated from *P. magnibaccum*. The identification of all compounds was achieved by physical properties: UV, IR, 1D/2D NMR and MS. These data were also confirmed by comparison with previously reported spectral data. The chemical structures of the isolated compounds as in Figure 1. Many of these constituents were previously isolated from *Piper* genus. Cepharanone A (**1**) has been isolated previously from *P. boehmeriaefolium*[18], while cepharadione A (**2**) from *P. betle*[19] and *P. nigrum*[20]. Besides, aristolactam AII (**3**) has been isolated previously from *P. wallichii*[21], 5,7-dimethoxyflavone (**4**) from *P. porphyrophyllum*[22] and piperumbellactam A (**7**) from *P. umbellatum*.[23]

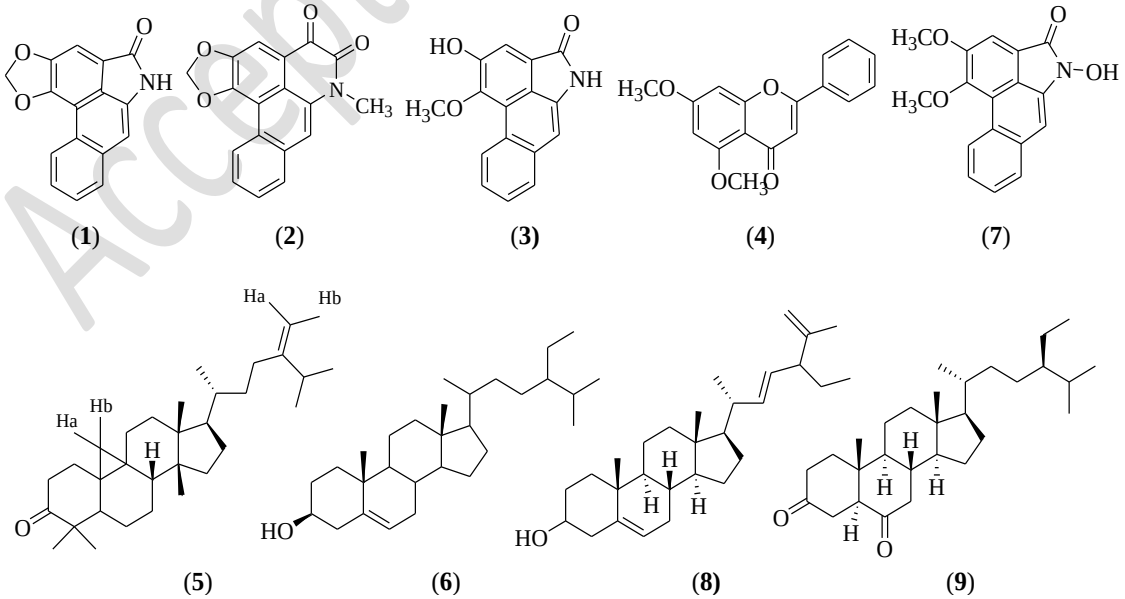

Figure 1. Phytochemicals isolated from *P. caninum* and *P. magnibaccum*

Cepharanone A (**1**) – Pale yellow needles (1.8 mg); m.p. 295-298°C; IR (ATR) υ_{max} cm^{-1}: ; 3072, 2925, 2228, 1688, 1691, 1615; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.53 (2H, s, OCH_2O), 7.19 (1H, s, H-9), 7.26 (s, $CDCl_3$), 7.60 (1H, s, H-2), 7.61 (2H, ddd, *J*=7.2, 7.2, 1.6 Hz, H-6 and H-7), 7.95 (1H, dd, *J*=7.6, 1.6 Hz, H-8), 9.80 (1H, s, N-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 103.4 (-OCH_2O), 104.4 (C-9), 105.1 (C-2), 121.4 (C-1), 124.8 (C-11), 125.3 (C-6), 135.0 (C-10), 127.7 (C-7), 127.0 (C-5), 129.0 (C-8), 126.0 (C-8a), 120.0 (C-5a), 135.6 (C-4a), 147.4 (C-4), 149.4 (C-3), 168.2 (C=O); EIMS *m/z* (% rel. int.): 263 [M^+, $C_{16}H_9NO_3$] (100), 235 (5), 207 (4), 177 (11), 131 (16).[24]

Cepharadione A (**2**) – Orange needles (3.1 mg); m.p. 350-354°C; IR (ATR) υ_{max} cm^{-1}: 1710, 1652; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.87 (3H, s, -NCH_3), 6.48 (2H, s, -OCH_2O-), 7.53 (1H, s, H-7), 7.69 (2H, m, H-9 and H-10), 7.92 (1H, m, H-8), 8.15 (1H, s, H-3), 9.01 (1H, m, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.5 (N-CH_3), 103.0 (-OCH_2O-), 109.0 (C-3), 114.2 (C-7), 115.2 (C-11b), 123.2 (C-3a), 125.5 (C-11a), 126.7 (C-10), 128.3 (C-8), 128.6 (C-11), 131.6 (C-7a), 132.4 (C-6a), 147.8 (C-2), 151.4 (C-1), 156.5 (C-5, C=O), 174.6 (C-4, C=O); EIMS *m/z* (% rel. int.): 305 [M^+, $C_{18}H_{11}NO_3$] (69), 277 (100), 248 (100), 232 (5), 163 (17).[25]

Aristolactam AII (**3**) – Pale yellow needles (0.7 mg); m.p. 269-272°C; IR (KBr) υ_{max} cm^{-1}: 3335, 2918, 1717, 1597, 1498; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.11 (3H, s, 4-OCH_3), 7.16 (1H, s, H-9), 7.28 (s, $CDCl_3$), 7.59 (2H, ddd, *J*=7.2, 7.2, 2.0 Hz, H-6 and H-7), 7.92 (1H, dd, *J*=6.8, 2.0 Hz, H-8), 7.71 (1H, s, H-2), 9.77 (1H, br.s, N-H), 9.20 (1H, dd, *J*=6.8, 2.0 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 59.5 (4-OCH_3), 104.0 (C-9), 113.3 (C-2), 120.1 (C-1), 122.4 (C-11), 125.2 (C-6), 127.0 (C-10), 127.1 (C-7), 127.3 (C-5), 129.0 (C-8), 133.9 (C-8a), 135.9 (C-5a), 141.3 (C-4a), 146.5 (C-4), 148.1 (C-3), 169.3 (C=O); EIMS *m/z* (% rel. int.): 265 [M^+, $C_{16}H_{11}NO_3$] (100), 251 (73), 222 (29).[26]

5,7-dimethoxyflavone (**4**) – White needle (6.3 mg); m.p. 158-159°C; IR (ATR) υ_{max} cm^{-1}: 2996, 1643, 1605, 1602, 1452, 1108; ^{1}H NMR (400 MHz, MeOD): δ 3.31 (s, MeOD), 3.89 (3H, s, 5-OCH_3), 3.97 (3H, s, 7-OCH_3), 6.63 (1H, s, H-3), 6.51 (1H, d, *J*=2.4 Hz, H-8), 6.81 (1H, d, *J*=2.4 Hz, H-6), 7.58 (3H, m, H-3', H-4', H-5'), 8.04 (2H, dd, *J*=7.6 and 3.8 Hz, H-2', H-6'); ^{13}C NMR (100 MHz, MeOD): δ 55.4 (7-OCH_3), 55.6 (5-OCH_3), 93.1 (C-6), 96.1 (C-8), 109.0 (C-3), 159.8 (C-4a), 125.9 (C-2', C-6'), 129.0 (C-3', C-5'), 131.1 (C-4'), 131.7 (C-1'), 159.9 (C-2), 160.6 (C-5), 161.0 (C-7), 164.2 (C-8a), 175.4 (C-4); EIMS (% rel int.): *m/z* 282 [M^+] (100), 252 (38), 180 (2), 102 (19).[27]

24-Methylenecycloartan-3-one (**5**) – Colourless needles (1.0 mg); m.p. 140-146ºC; IR (ATR) υ_{max} cm^{-1}: 3050, 2990, 1645, 1608, 1602, 1430; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.60 (1H, d, *J*=4.0 Hz, H-19a), 0.81 (1H, d, *J*=4.0 Hz, H-19b), 0.91 (1H, m, H-6b), 0.92 (3H, d,

J=2.0 Hz, 21-CH_3), 0.93 (3H, s, 29-CH_3), 1.02 (3H, s, 18-CH_3), 1.04 (3H, d, *J*=2.4 Hz, 26-CH_3), 1.06 (3H, d, *J*=2.4 Hz, 27-CH_3), 1.07 (3H, s, 30-CH_3), 1.12 (3H, s, 28-CH_3), 1.19 (1H, m, H-11b), 1.23 (2H, m, H-22), 1.35 (1H, m, H-7a), 1.39 (2H, m, H-23), 1.40 (1H, m, H-16b), 1.56 (1H, m, H-6a), 1.58 (1H, m, H-17), 1.63 (1H, m, H-5), 1.67 (1H, m, H-1b), 1.69 (2H, m, H-12), 1.73 (1H, m, H-16a), 1.85 (1H, m, H-8), 1.89 (2H, m, H-15), 1.91 (1H, m, H-1a), 1.97 (1H, m, H-7b), 2.08 (1H, m, H-11a), 2.12 (1H, m, H-20), 2.33 (1H, m, H-2a), 2.73 (1H, ddd, *J*=6.4, 7.2, 14.0 Hz, H-2b), 4.69 (1H, s, H-31a), 4.74 (1H, s, H-31b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.1 (18-CH_3), 18.2 (21-CH_3), 19.2 (30-CH_3), 20.6 (28-CH_3), 21.0 (C-9), 21.5 (C-6), 21.7 (27-CH_3), 22.0 (26-CH_3), 22.1 (29- CH_3), 25.8 (C-16), 25.9 (C-10), 26.8 (C-11), 28.1 (C-7), 29.5 (C-19), 31.3 (C-23), 31.3 (C-12), 33.4 (C-1), 33.8 (C-25), 34.9 (C-15), 35.5 (C-22), 36.3 (C-20), 37.4 (C-2), 45.4 (C-13), 47.8 (C-8), 48.4 (C-5), 48.7 (C-14), 50.2 (C-4), 52.3 (C-17), 105.9 (C-31), 156.8 (C-24), 216.5 (3-C=O); GCMS *m/z* (% rel. int.): 438 [M^+, $C_{31}H_{50}O$] (83), 423 (59), 395 (46), 313 (71), 271 (24).[28]

β-Sitosterol (**6**) – White crystalline needles (11.8 mg); m.p. 130-135ºC; IR (KBr) υ_{max} cm^{-1}: 3435, 2966, 1461; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.71 (3H, s, H-18), 0.76 (3H, d, *J*=6.4 Hz, H-27), 0.82 (3H, d, *J*=6.4 Hz, H-26), 0.87 (3H, t, *J*=3.8 Hz, H-29), 0.93 (3H, t, *J*=3.8 Hz, H-21), 1.03 (3H, s, H-19), 1.05-2.31 (m, -CH_2-) 3.53 (1H, m, H-3), 5.37 (1H, d, *J*=5.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.8 (C-29), 11.9 (C-18), 18.7 (C-21), 19.0 (C-27), 19.3 (C-19), 19.8 (C-26), 21.0 (C-11), 23.0 (C-28), 24.3 (C-15), 26.0 (C-23), 28.2 (C-7), 29.1 (C-25), 31.6 (C-2), 31.9 (C-8, C-16), 33.9 (C-22), 36.1 (C-20), 36.5 (C-10), 37.2 (C-1), 39.7 (C-12), 42.3 (C-4), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5); EIMS *m/z* (rel. int.): 414 (100) [M^+, $C_{29}H_{50}O$].[29]

Piperumbellactam A (**7**) – Yellow needle (5.4 mg); m.p. 208-210°C; IR (ATR) υ_{max} cm^{-1}: 3156, 3017, 2951, 1710, 1652; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.11 (3H, s, 4-OCH_3), 4.15 (3H, s, 3-OCH_3), 7.11 (1H, s, H-9), 7.60 (2H, m, H-6 and H-7), 7.83 (1H, dd, *J*=6.4, 2.4 Hz, H-8), 7.85 (1H, s, H-2), 8.06 (1H, br.s, N-OH), 9.27 (1H, dd, *J*=6.4, 2.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 57.0 (4-OCH_3), 60.4 (3-OCH_3), 105.6 (C-9), 110.0 (C-2), 121.1 (C-1), 124.4 (C-11), 127.6 (C-6), 127.0 (C-5), 127.6 (C-6), 129.0 (C-8), 134.0 (C-8a), 126.0 (C-5a), 141.3 (C-4a), 151.6 (C-4), 154.6 (C-3), 169.1 (C=O); EIMS *m/z* (% rel. int.): 295 [M^+, $C_{17}H_{13}NO_4$] (69), 279 (25), 250 (40), 237 (100), 223 (17), 194 (18), 165 (18), 152 (12).[23]

24*S*-ethylcholesta-5,22,25-trien-3β-ol (**8**) - White solid (32.4 mg); m.p. 140-146ºC; IR (KBr) υ_{max} cm^{-1}: 3407, 2958, 2853, 1459; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.72 (3H, m, 18-CH_3), 0.85 (3H, t, *J*=7.6 Hz, 29-CH_3), 0.94 (1H, m, H-9), 1.03 (3H, s, 19-CH_3), 1.04 (3H, s, H-21), 1.08 (1H, m, H-14), 1.09 (1H, m, H-1b), 1.15 (2H, m, H-12), 1.19 (1H, m, H-17), 1.20 (1H, m,

H-16b), 1.27 (2H, m, H-28), 1.46 (1H, m, H-20), 1.49 (1H, m, H-11a), 1.52 (1H, m, H-15a), 1.55 (1H, m, H-16a), 1.56 (1H, m, H-1a), 1.59 (2H, m, H-2), 1.67 (3H, s, 27-CH_3), 1.85 (1H, m, H-8), 1.99 (2H, m, H-7), 2.44 (1H, q, *J*=7.6 Hz, H-24), 3.55 (1H, m, H-3), 4.72 (2H, br d, *J*=2.0 Hz, H-26), 5.21 (1H, dd, *J*=7.6 and 13.2 Hz, H-23), 5.24 (1H, dd, *J*=7.6 and 13.2 Hz, H-22), 5.37 (1H, t, *J*=5.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.0 (C-18), 20.8 (19-CH_3), 12.1 (29-CH_3), 19.4 (C-21), 20.2 (C-27), 20.8 (C-19), 21.1 (C-11), 24.3 (C-15), 25.7 (C-16), 25.8 (C-28), 31.9 (C-2), 31.7 (C-7), 31.9 (C-8), 36.5 (C-10), 37.3 (C-1), 39.7 (C-12), 40.2 (C-20), 42.3 (C-13), 42.3 (C-4), 50.2 (C-9), 52.0 (C-24), 56.0 (C-17), 56.9 (C-14), 71.8 (C-3), 109.5 (C-26), 121.7 (C-6), 130.1 (C-23), 137.2 (C-22), 140.8 (C-5), 148.6 (C-25); GCMS *m/z* (% rel. int.): 410 [M^+, $C_{29}H_{46}O$] (40), 392 (18), 273 (40), 271 (100), 255 (60).[30]

Stigmast-3,6-dione (**9**) – White needles (13.1 mg); m.p. 194-196°C; IR (KBr) υ_{max} cm^{-1}: 2924, 2864, 1708; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.71 (3H, s, 18-CH_3), 0.81 (3H, d, *J*=6.8 Hz, H-27), 0.85 (3H, d, *J*=6.8 Hz, H-26), 0.86 (3H, t, *J*=6.8 Hz, H-29), 0.98 (3H, d, *J*=6.8 Hz, H-21), 1.04 (3H, s, 19-CH_3), 1.15 (2H, m, H-23), 1.32 (1H, H-22 (H-b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.9 (C-29), 12.0 (C-18), 12.6 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 21.7 (C-11), 23.1 (C-28), 24.0 (C-15), 26.1 (C-23), 28.0 (C-16), 29.2 (C-25), 33.9 (C-22), 36.1 (C-20), 37.0 (C-4), 37.4 (C-2), 38.0 (C-1), 38.1 (C-8), 39.4 (C-12), 41.3 (C-10), 43.0 (C-13), 46.6 (C-7), 45.8 (C-24), 53.5 (C-9), 56.0 (C-17), 56.6 (C-14), 57.5 (C-5), 209.1 (C-6), 211.2 (C-3), GCMS *m/z* (% rel. int.): 428 [M^+, $C_{29}H_{46}O$] (100), 399 (18), 287 (32), 245 (62).[31]

In the present study, the extracts of *P. caninum* and *P. magnibaccum* were subjected to the antityrosinase assay. The extract of *P. caninum* and *P. magnibaccum*; the selected compounds which were two aporphine alkaloids, (**2**) and (**7**) were also screened in this assay. The positive control used in mushroom tyrosinase assay was kojic acid as it has been understood to act as a potent tyrosinase inhibitor.[32] Table 1 summaries the results of mushroom tyrosinase inhibition of the tested samples.

Table 1. Tyrosinase inhibitory activity of *P. caninum* and *P. magnibaccum*

Samples	Parts	Extracts	Tyrosinase Inhibition (I%)
P. caninum	Aerial part	Hexane	39.61 ± 0.6
		Dichloromethane	42.80 ± 0.1
P. magnibaccum	Leaves	Hexane	30.81 ± 0.8
		Ethyl acetate	48.35 ± 0.6
		Methanol	41.02 ± 0.1
	Stem	Hexane	27.84 ± 0.8
		Methanol	28.01 ± 0.8
Cepharadione A (**2**)			40.84 ± 0.9
Piperumbellactam (**7**)			28.16 ± 0.5
Kojic acid			95.80 ± 0.1

I% - Percentage inhibition

Ethyl acetate extracts from leaves of *P. magnibaccum* gave the highest inhibition value at 48.35% followed by DCM extracts of *P. caninum*, 42.80%. Meanwhile, the tested compounds displayed weak tyrosinase activity compared to the positive control, kojic acid which inhibited mushroom tyrosinase activity at 91.3%. One of the naturally isolated compounds notably used as tyrosinase inhibitor in the cosmetic industry is oxyresveratrol and, particularly from *Morus alba*. These phenolics compounds possessed four and three numbers of hydroxyl substituents to act as tyrosine inhibitor.[33] Compounds with *N*-substituted either as phenanthrene chromophore (**2**) and (**7**) did not influence the tyrosinase inhibition.[34] Previously, *P. nigrum* and *P. longum* have shown strong tyrosinase inhibitory activity.[35-36] Piperlonguminine isolated from *P. longum* was found to inhibit melanin production in melanoma B16 cells stimulated with α-melanocyte stimulating hormone (α-MSH), 3-isobutyl-1-methylxanthine or protoporphyrin IX, with stronger depigmenting efficacy than kojic acid.[37] In addition, asebogenin, a dihydrochalcone isolated from *P. elongatum* exhibited strong tyrosinase activity and had almost the same activity to that of kojic acid.[38]

Conclusion

In conclusion, our study led to the isolation and identification of nine compounds from *P. caninum* and *P. magnibaccum*, and compound (**2**) exhibited moderate tyrosinase inhibitory activity. These findings support further research to investigate the mechanism of an isolated

compound from the extract for better understanding as a potential inhibitor for tyrosinase and may be of interest to clarify the physiological role of the enzyme.

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Conflict of interests

The authors claim that there is no conflict of interest.

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