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QUASSINOIDS FROM THE ROOTS OF *Brucea javanica* AND THEIR POTENTIAL CYTOTOXICITY

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ABSTRACT

A phytochemical study of the roots of *Brucea javanica* was conducted and four alkaloids and three quassinoids were isolated. The structures of the compounds were verified using physical and spectroscopic data, which were compared to the literature. The cytotoxicity of the compounds was evaluated against KB (oral cavity carcinoma), NCI-H187 (small cell lung cancer), and MCF-7 (breast adenocarcinoma) cell lines. The results showed that the quassinoid, Bruceine A, exhibited very strong cytotoxicity against KB, and high potency against NCI-H187, with both being 581- and 100-fold more active than ellipticine, and 99- and 10-fold more active than doxorubicin, respectively. These results suggest the potential use of bruceine A as an anticancer agent.

Keywords: *Brucea javanica,* Alkaloids, Quassinoids, Cytotoxicity, Anticancer

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INTRODUCTION

Brucea javanica (L.) Merr. (Simaroubaceae), as Rat Cha Dat in Thai, has a long history of traditional use for the treatment of conditions such as hemorrhoids, chronic dysentery, diarrhea, and amebiasis.[1,2] Phytochemical studies of *B. javanica* have previously identified the presence of quassinoids, quassinoid glycosides, apotirucallane-type triterpenoids, lignans, alkaloids, and alkaloid glycosides.[3-16] Compounds extracted from *B. javanica* have displayed several bioactivities, including anti-inflammatory, anti-tuberculosis, antiplasmodial, antimycobacterial, cytotoxic and antileukemic, antibabesial activities, hypoglycemic effect, and antitrypanosomal activity.[2,4,6,7,17-20] Our preliminary survey of the bioactive components of *B. javanica* revealed that crude EtOAc and MeOH extracts displayed potent cytotoxicity against human small cell lung cancer (NCI-H187), human oral cavity carcinoma (KB), and human breast adenocarcinoma (MCF-7) cells. Hence, the objective of this study was to isolate, structurally characterize, and evaluate the anti-KB, NCI-H187, and MCF-7 activities of compounds present in the roots of *B. javanica*.

EXPERIMENTAL

General Procedure

The current study utilized analytical techniques and materials that are consistent with those previously reported in the literature.[21] These techniques included infrared spectroscopy (IR), Nuclear magnetic resonance spectroscopy (NMR), and High-resolution time-of-flight mass spectrometry (HRTOFMS) for characterization purposes, as well as melting point analysis and optical rotation measurements. Chromatographic materials employed in this study were of the same type as those used in previous studies.

Plant Materials

Roots of *Brucea javanica* were collected from Phutthaisong district, Buriram province, Thailand in July 2015 and subsequently air-dried. A voucher specimen (designated as Apichart Suksamrarn, No. 087) has been deposited at the Faculty of Science, Ramkhamhaeng University for reference and authentication purposes.

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Extraction and Isolation

In this study, 6 kg of air-dried *B. javanica* roots were ground and sequentially extracted with n-hexane, ethyl acetate (EtOAc), and methanol (MeOH). The resulting n-hexane, EtOAc, and MeOH extracts were obtained in yields of 10.31 g, 64.61 g, and 97.77 g, respectively, after removal of the solvents using a rotary evaporator at temperatures of 40–45°C. Upon observing significant cytotoxic activities in the EtOAc and MeOH extracts, these extracts were subjected to further investigation for active substances. The EtOAc extract (97.0 g) was fractionated through column chromatography using solvents of increasing polarity, including n-hexane, n-hexane-EtOAc, EtOAc, EtOAc-MeOH, and MeOH, resulting in 10 fractions (E_1-E_{10}). Fraction E_6 (9.77 g) was further separated into 4 fractions (E_{6a}-E_{6d}) using Sephadex LH-20 eluted with MeOH. The final fraction, E_{6c}, was purified using isocratic (n-hexane-EtOAc, 60:40) column chromatography, yielding compounds **1** (61.2 mg) and **2** (37.2 mg). Fraction E_7 (6.39 g) was chromatographed on Sephadex LH-20 with MeOH, resulting in 5 fractions (E_{7a}-E_{7e}). Compound **3** (55.1 mg) was obtained from fraction E_{7c} after repeated recrystallization with EtOAc, and compound **4** (1.6 mg) was obtained from fraction E_{7e} in a similar manner. Fraction E_8 (10.22 g) was eluted four times with MeOH through Sephadex LH-20 to give compound **5** (35.5 mg). The MeOH extract (97.0 g) was chromatographed on a silica column (silica gel 60, particle size 0.063-0.200 mm, Merck) using solvents of increasing polarity, including n-hexane, n-hexane-EtOAc, and EtOAc, resulting in 8 fractions (M_1-M_8). Fraction M_6 (20.0 g) was further purified using Sephadex LH-20 column chromatography with methanol elution three times, followed by elution with a 5% MeOH in CH_2Cl_2 solution on a silica column, yielding compounds **6** (133.9 mg) and **7** (57.8 mg).

Cytotoxic Activity

The cytotoxic activity of the isolated compounds was evaluated using the modified resazurin microplate assay (REMA) on three cell lines: human breast adenocarcinoma (MCF-7), human small cell lung carcinoma (NCI-H187) and human oral cavity carcinoma (KB).[22]

RESULTS AND DISCUSSION

Extraction and Isolation of *Brucea javanica* Roots

Column chromatography of EtOAc and MeOH extracts from *Brucea javanica* roots, active against NCI-H187, KB, and MCF-7 cells, led to the isolation of four alkaloids (1-hydroxy-11-methoxycanthin-6-one (**1**), 11-hydroxy-1-methoxycanthin-6-one (**2**), 11-hydroxycanthin-6-one (**3**), and 5-methoxy-can thin-6-one (**4**)) and three quassinoids (bruceine A (**5**), bruceine D (**6**), and yadanziolide A (**7**)) as depicted in Fig.-1.

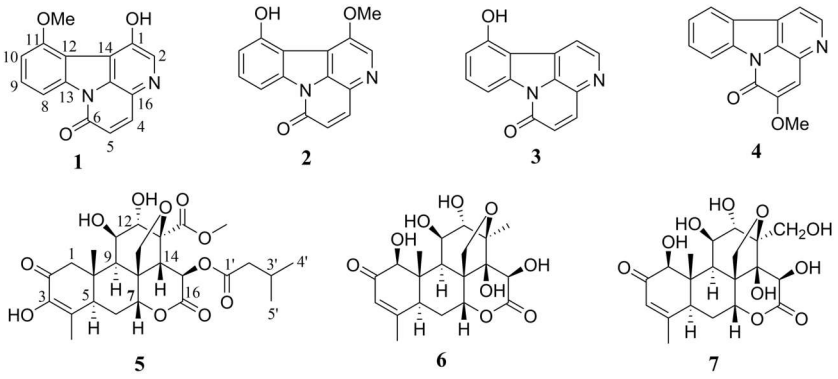

Fig.-1**:** Structures of Compounds **1**–**7** Isolated from the Roots of *Brucea javanica*

1-Hydroxy-11-methoxycanthin-6-one (1)

Pale yellow amorphous solid; IR ν_{max} 3309, 2981, 2828, 671, 1636, 1595, 1493, 1428, 1399, 1352, 1130, 1065, 989 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.74 (1H, s, 1-OH), 8.43 (1H, s, H-2), 8.32 (1H, d, J = 8.1 Hz, H-8), 7.96 (1H, d, J = 9.7 Hz, H-4), 7.61 (1H, t, J = 8.1 Hz, H-9), 6.99 (1H, d, J = 8.1 Hz, H-10), 6.77 (1H, d, J = 9.7 Hz, H-5), 4.18 (3H, s, 11-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 160.3 (C-6), 153.0 (C-11), 148.4 (C-1), 139.6 (C-13), 139.3 (C-4), 135.9 (C-2), 132.9 (C-15), 131.5 (C-9), 128.9 (C-16), 124.2

(C-5), 114.1 (C-14), 113.0 (C-12), 111.4 (C-8), 106.9 (C-10), 56.7 (C-11-OCH_3); HRTOFMS (ESI^+): m/z 267.0758 $[M + H]^+$ (calcd. For $C_{15}H_{11}N_2O_3$, 267.0764).

11-Hydroxy-1-methoxycanthin-6-one (2)
Pale yellow amorphous solid; IR ν_{max} 3368, 1671, 1630, 1601, 1504, 1428, 1396, 1349, 1311, 1250, 1121, 986 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.25 (1H, s, 11-OH), 8.48 (1H, s, H-2), 8.16 (1H, d, *J* = 8.0 Hz, H-8), 7.95 (1H, d, *J* = 9.7 Hz, H-4), 7.56 (1H, t, *J* = 8.0 Hz, H-9), 6.99 (1H, d, *J* = 8.0 Hz, H-10), 6.86 (1H, d, *J* = 9.7 Hz, H-5), 4.33 (3H, s, 1-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.8 (C-6), 152.4 (C-11), 148.4 (C-1), 139.6 (C-13), 138.6 (C-4), 132.7 (C-9), 132.3 (C-15), 131.3 (C-16), 129.5 (C-2), 126.6 (C-5), 117.1 (C-14), 112.6 (C-10), 111.0 (C-12), 108.8 (C-8), 57.8 (C-1-OCH_3); HRTOFMS (ESI^+): m/z 267.0757 $[M + H]^+$ (calcd. For $C_{15}H_{11}N_2O_3$, 267.0764).

11-Hydroxycanthin-6-one (3)
Yellow solid; IR ν_{max} 3052, 1671, 1639, 1469, 1349, 1299, 1247, 1127, 980 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ = 8.74 (1H, d, *J* = 5.0 Hz, H-2), 8.17 (1H, d, *J* = 5.0 Hz, H-1), 8.07 (1H, d, *J* = 9.7 Hz, H-4), 8.02 (1H, d, *J* = 8.0 Hz, H-8), 7.56 (1H, t, *J* = 8.0 Hz, H-9), 6.97 (1H, br d, *J* = 9.7 Hz, H-5) 6.96 (1H, d, *J* = 8.0 Hz, H-10); ^{13}C NMR (CD_3OD, 100 MHz): δ = 161.7 (C-6), 157.6 (C-11), 147.5 (C-2), 142.6 (C-13), 140.8 (C-4), 136.6 (C-16), 134.2 (C-9), 132.3 (C-15), 129.9 (C-5), 122.9 (C-14), 120.3 (C-1), 113.8 (C-12), 113.6 (C-10), 109.4 (C-8); HRTOFMS (ESI^+): m/z 237.0668 $[M + H]^+$ (calcd. For $C_{14}H_9N_2O_2$, 237.0658).

5-Methoxy-canthin-6-one (4)
Yellow amorphous solid; IR ν_{max} 3338, 2940, 2890, 1665, 1636, 1604, 1589, 1472, 1288, 1130, 948 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ = 8.73 (1H, d, *J* = 5.3 Hz, H-2), 8.61 (1H, d, *J* = 8.2 Hz, H-11), 8.29 (1H, d, *J* = 7.7 Hz, H-8), 8.13 (1H, d, *J* = 5.3 Hz, H-1), 7.78 (1H, br t, *J* = 8.2 Hz, H-10), 7.61 (1H, br t, *J* = 7.7 Hz, H-9), 7.29 (1H, s, H-4), 4.09 (3H, s, 5-OCH_3); ^{13}C NMR (CD_3OD, 100 MHz): δ = 157.4 (C-6), 157.2 (C-5), 146.2 (C-2), 141.2 (C-12), 137.9 (C-16), 132.8 (C-10), 132.5 (C-14), 129.4 (C-15), 128.0 (C-9), 126.9 (C-13), 125.1 (C-8), 118.6 (C-11), 116.2 (C-1), 109.7 (C-4); HRTOFMS (ESI^+): m/z 273.0637 $[M + Na]^+$ (calcd. For $C_{15}H_{10}N_2NaO_2$, 237.0634).

Bruceine A (5)
White solid; IR ν_{max} 3426, 2964, 2858, 1733, 1671, 1642, 1440, 1390, 1361, 1118, 980 cm^{-1}; ^1H NMR (Pyridine-d_6, 400 MHz): δ = 5.97 (1H, br s, H-5), 5.11 (1H, overlapping signal, H-12), 5.07 (1H, overlapping signal, H-7), 5.04 (1H, overlapping signal, H-20b), 4.76 (1H, br t, *J* = 7.6 Hz, H-11), 3.91 (1H, d, *J* = 6.8 Hz, H-20a), 3.81 (3H, s, H-22), 3.26 (1H, d, *J* = 15.9 Hz, H-1b), 2.57 (1H, d, *J* = 4.3 Hz, H-9), 2.45 (1H, d, *J* = 15.9 Hz, H-1a), 2.33 (2H, br t, *J* = 8.2 Hz, H-2′), 2.26 (1H, dt, *J* = 14.7, 2.5 Hz, H-6β), 2.14-2.22 (1H, m, H-3′), 1.93 (3H, br s, H-18), 1.71 (1H, ddd, *J* = 14.7, 11.2, 2.5 Hz, H-6α), 1.62 (3H, br s, H-19), 0.94 (3H, d, *J* = 6.6 Hz, H-4′), 0.91 (3H, d, *J* = 6.6 Hz, H-5′); ^{13}C NMR (Pyridine-d_6, 100 MHz): δ = 193.1 (C-2), 171.3 (C-21, C-1′), 168.3 (C-16), 146.0 (C-3), 128.3 (C-4), 83.8 (C-7), 82.7 (C-13), 75.6 (C-12), 73.1 (C-11), 73.7 (C-20), 68.4 (C-15), 52.4 (C-22), 50.7 (C-14), 50.1 (C-1), 46.2 (C-8), 43.3 (C-2′), 42.4 (C-5, C-9), 41.3 (C-10), 29.5 (C-6), 25.9 (C-3′), 22.5 (C-4′), 22.4 (C-5′), 15.7 (C-19), 13.4 (C-18); HRTOFMS (ESI^+): m/z 545.1981 $[M + Na]^+$ (calcd. For $C_{26}H_{34}NaO_{11}$, 545.1993).

Bruceine D (6)
White solid; IR ν_{max} 3397, 2876, 1706, 1659, 1434, 1373, 1264, 1159, 1074 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ = 6.03 (1H, br s, H-3), 5.20 (1H, s, H-15), 5.09 (1H, br s, H-11), 4.57 (1H, d, *J* = 4.5 Hz, H-12), 4.51 (1H, d, *J* = 7.4 Hz, H-20b), 4.22 (1H, br s, H-1), 3.81 (1H, d, *J* = 7.4 Hz, H-20a), 3.74 (1H, br s, H-7), 2.93 (1H, br d, *J* = 12.8 Hz, H-5), 2.37 (1H, d, *J* = 3.6 Hz, H-9), 2.33 (1H, dt, *J* = 14.9, 6.4 Hz, H-6b), 1.95 (3H, s, H-18), 1.82 (1H, ddd, *J* = 14.9, 12.8, 2.0 Hz, H-6a), 1.41 (3H, s, H-21), 1.17 (3H, s, H-19); ^{13}C NMR (CD_3OD, 100 MHz): δ = 200.3 (C-2), 176.7 (C-16), 166.0 (C-4), 125.6 (C-3), 85.4 (C-14), 83.5 (C-1), 81.9 (C-7), 81.6 (C-11), 76.9 (C-13), 75.9 (C-12), 71.1 (C-15), 70.8 (C-20), 51.2 (C-8), 49.4 (C-10), 46.7 (C-9), 44.9 (C-5), 29.1 (C-6), 23.0 (C-18), 18.9 (C-21), 11.9 (C-19); HRTOFMS (ESI^+): m/z 433.1469 $[M + Na]^+$ (calcd. For $C_{20}H_{26}NaO_9$, 433.1469).

Yadanziolide A (7)

White solid; IR ν_{max} 3379, 1730, 1659, 1431, 1376, 1261, 1153, 1083 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ = 6.02 (1H, br s, H-3), 5.22 (1H, s, H-15), 5.09 (1H, t, *J* = 2.8 Hz, H-11), 4.57 (1H, br s, H-12), 4.55 (1H, d, *J* = 7.2 Hz, H-20b), 4.23 (1H, br s, H-1), 4.17 (1H, d, *J* = 11.8 Hz, H-21b), 3.91 (1H, d, *J* = 7.2 Hz, H-20a), 3.89 (1H, d, *J* = 11.8 Hz, H-21a), 3.86 (1H, br s, H-7), 2.94 (1H, br d, *J* = 11.8 Hz, H-5), 2.41 (1H, d, *J* = 2.8 Hz, H-9), 2.34 (1H, dt, *J* = 14.9, 2.8 Hz, H-6b), 1.96 (3H, br s, H-18), 1.82 (1H, ddd, *J* = 14.9, 11.8, 2.8 Hz, H-6a), 1.16 (3H, br s, H-19); ^{13}C NMR (CD_3OD, 100 MHz): δ = 200.3 (C-2), 176.6 (C-16), 166.0 (C-4), 125.6 (C-3), 85.1 (C-14), 84.2 (C-13), 83.4 (C-1), 81.1 (C-11), 78.1 (C-7), 75.9 (C-12), 71.6 (C-20), 71.2 (C-15), 64.9 (C-21), 51.6 (C-8), 49.9 (C-10), 46.4 (C-9), 44.9 (C-5), 29.1 (C-6), 23.0 (C-18), 11.9 (C-19); HRTOFMS (ESI^+): m/z 449.1410 $[M + Na]^+$ (calcd. For $C_{20}H_{26}NaO_{10}$, 449.1418).

The structures of all known compounds were confirmed by comparison of physical data and spectroscopic evidence with literature values.[16, 23-28]

Cytotoxic Activity of Isolated Compounds

The cytotoxicity of all compounds on KB, NCI-H187, and MCF-7 cell lines is presented in Table-1.

Table-1**:** Cytotoxic Activities of Compounds **1**–**7** Isolated from the Roots of *Brucea javanica*

Compound	Cytotoxicity (IC_{50}, μM)		
	KB	NCI-H187	MCF-7
1	46.16	NA	49.46
2	NA	NA	NA
3	NA	NA	NA
4	NT	NT	NT
5	0.01	0.05	NA
6	2.68	0.65	NA
7	4.08	3.31	13.87
Ellipticine	5.81	4.99	NT
Doxorubicin	0.99	0.49	13.26
Tamoxifen	NT	NT	13.45

NA = inactive at 50 μg/mL, NT = not tested.

Among the compounds tested, quassinoids **5**-**7** displayed the most pronounced cytotoxic activity against both KB and NCI-H187 cell lines. Specifically, compound **7** demonstrated the strongest potency towards the MCF-7 cell line. In contrast, compounds **1**-**3** were either less active or inactive against all three cell lines, while the activity of compound **4** was found to be weak. Of note, the cytotoxic activity of compound **5** against KB cells was particularly robust, 581- and 99-fold greater than ellipticine and doxorubicin, respectively. Additionally, compound **5** displayed potent activity against NCI-H187 cell lines, 100- and 10-fold greater than ellipticine and doxorubicin, respectively. Quassinoid **7** displayed selective activity against the MCF-7 cell line, comparable to doxorubicin and tamoxifen, while quassinoids **5** and **6** were inactive. Preliminary structure-activity analysis suggests that the presence of an ester group and an additional hydroxyl group at C-3 in compound **5** may contribute to its enhanced activity against KB and NCI-H187 cell lines. Similarly, the presence of a hydroxymethyl group at position C-13 in quassinoid **7** may have contributed to its activity against the MCF-7 cell line in comparison to compound **6**. Based on the results, it can be concluded that brucine A (compound **5**) should be considered as a potential lead molecule for further development as an anticancer therapeutic.

CONCLUSION

In conclusion, this study investigated the phytochemistry of the roots of *Brucea javanica* and identified seven compounds, including four alkaloids and three quassinoids. The results demonstrated that among the isolated compounds, quassinoid **5** displayed the most potent cytotoxicity against KB and NCI-H187 cell lines, with IC_{50} values of 0.01 and 0.05 μM, respectively. Furthermore, compound **7** showed selective cytotoxicity towards the MCF-7 cell line, with an IC_{50} value of 13.87 μM. These findings contribute to the growing body of evidence suggesting that *Brucea javanica* has the potential as a source of anticancer compounds. Further

research is necessary to investigate the mechanisms behind the cytotoxicity of these compounds, which could lead to the development of new and more effective cancer treatments. The results of this study demonstrate the importance of natural product discovery in drug development and highlight the potential of *Brucea javanica* as a valuable resource for the discovery of novel anticancer agents.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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