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Two new alkaloids isolated from traditional Chinese medicine Binglang the fruit of *Areca catechu*

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ABSTRACT

Binglang, the fruit of *Areca catechu* L, has a long history as an important Chinese herbal medicine. Two new alkaloids (**1** and **2**), along with forty-one known compounds (**3–43**) were isolated from the dried fruit of *Areca catechu* L. The structures were elucidated on basis of the IR, UV, MS and 1D, 2D NMR spectroscopic data. Compounds **26** and **33** showed weak cytotoxicity against human gastric cancer cell line (BGC-823) with IC_{50} of 15.91 μM and 20.13 μM, respectively.

1. Introduction

Areca catechu L, called “Binglang” in Chinese, belongs to the family Arecaceae which is comprised of fifteen species. This plant is widely distributed in southern and southeast Asia including China, India, Pakistan, Indonesia, Malaysia, Philippines and New Guinea. In Hainan province of China for its important economic and medicinal benefits [1,2]. The fruits of *A. catechu* (frequently known as areca nut) are popular chewable items in Asian countries [3,4]. In addition, the peel and seed of *A. catechu* have been used as traditional Chinese medicine (TCM) since 1700 years [5]. Since 1953, the areca seed heve been listed in the Pharmacopoeia of the People's Republic of China and over 100 prescriptions containing areca seed such as Binglang sixiao pill, Simo decoction, and Jianweixiaoshi tablets have been utilized for treatment of parasitic diseases, abdominal distension, abdominal pain, and jaundice [6]. Dafupi, which is the peel of *A. catechu*, is used as TCM to treat ascites and edema [6,7].

The major chemical constituents in *A. catechu* have earlier been identified are; alkaloids, flavonoids, tannins, triterpenes, and fatty acids [2,8]. A number of pharmacological activities such as anti-parasitic effect, anti-depressive effect, anti-fatigue effect, anti-oxidant effect and increasing gastrointestinal motility properties [9,10]. The alkaloids of *A. catechu* were found to have anti-taeniasis, anti-tumor, antibacterial, and antifungal effects [8]. Chemical constituents of *A. catechu*, including catechin, chrysoeriol, isorhamnetin, luteolin, isovanillic acid, protocatechuicacid, showed significant antioxidant activity [2,4,8]. These pharmacological activities indicated that the *A. catechu* have a promising future for treating diseases, particular parasitosis, digestive system diseases and nervous system disorders.

In order to find structurally unique and bioactive natural products, 95% EtOH extract of this plant were separated, which led to the isolation of two new compounds, named as acatechu A (**1**) and acatechu B (**2**) (Fig. 1), along with forty-one known ones (**3–43**). Compounds **7–11**, **13–14**, **16–17**, **23–27**, **34–35**, **40**, and **42–43** were isolated from this plant for the first time. In addition, all isolated compounds were evaluated for their cytotoxic activity against four human cancer cell lines (Hela, BGC-823, Hep G2 and HCT-116). Compounds **26** and **33** showed weak cytotoxicity against human gastric cancer cell line BGC-823.

2. Results and discussion

Compound **1** was obtained as yellowish power. The molecular formula of **1** was $C_{20}H_{27}NO_7$ determined by its negative HR-ESI-MS *m/z* 416.1673 $[M\text{-}Na]^-$ (calcd for $C_{20}H_{27}NO_7{}^-$, 416.1685). The UV spectrum of **1** showed the absorption maxima at 206 and 279 nm. The IR spectrum indicated the presence of hydroxyl (3420 cm^{-1}), carbonyl

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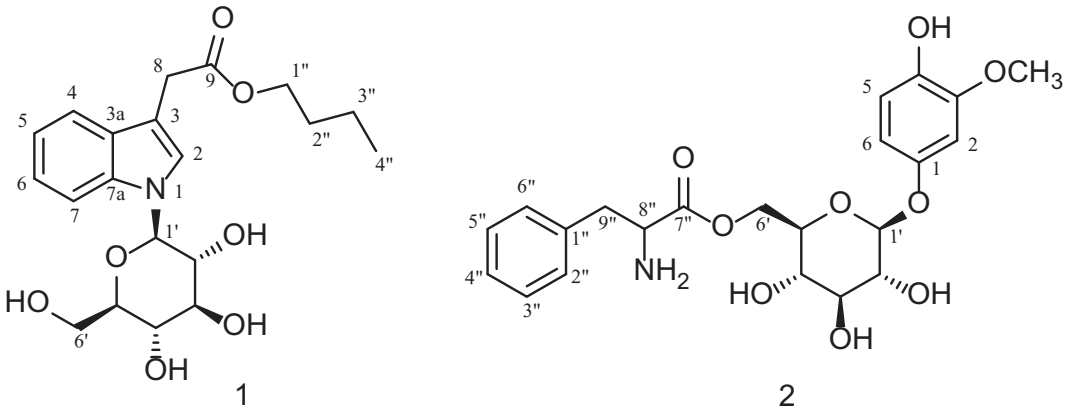

Fig. 1. The chemical structures of **1–2**.

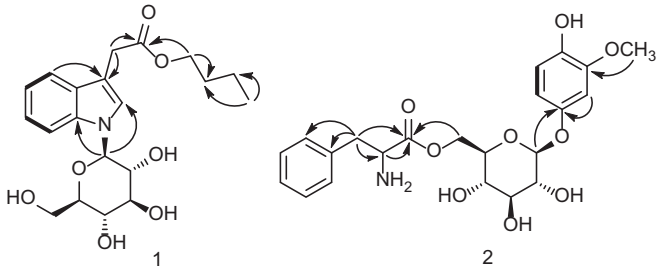

Fig. 2. The key ^{1}H-^{1}H COSY (—) and HMBC (↷) correlations of **1–2.**

(1717 cm^{-1}), and aromatic ring (1576 and 1463 cm^{-1}). The ^{1}H NMR spectrum showed four aromatic proton signals [δ_H 7.54 (1H, d, J = 8.3, H-7), 7.52 (1H, d, J = 7.9, H-4), 7.19 (1H, ddd, J = 8.2, 7.2, 1.1, H-6), 7.09 (1H, m, H-5)]. The proton signals in the region of 5.44 ppm–3.50 ppm [δ_H 5.44 (1H, d, J = 9.1, H-1′), 3.92 (1H, m, H-2′), 3.60 (1H, t, J = 9.0, H-3′), 3.57 (1H, dd, J = 5.7, 2.2, H-5′), 3.50 (1H, m, H-4′), 3.87 (1H, d, J = 2.0, H_1–6′), 3.71 (1H, dd, J = 12.2, 5.7, H_2–6′] suggested the presence of a sugar moiety, which was identified as glucopyranoside based on analysis of HMBC and ^{1}H-^{1}H COSY NMR data (Fig. 2). In ^{13}C NMR spectrum signals for a carbonyl groups [δ_C 174.3C-9], a benzene ring [δ_C 138.4C-7a, 129.7C-3a, 123.1C-6, 120.9C-5, 119.8C-4, 111.6C-7], and a glucopyranoside unit [δ_C 86.7C-1′, 80.6C-3′, 78.9C-5′, 73.8C-2′, 71.5C-4′, 62.8C-6′] were observed. The ^{1}H and ^{13}C NMR spectra of **1** were closely similar to those of 3-carboxymethyl indole 1-*N*-*β*-D-glucopyranoside [11], with the only difference of an extra signal for *n*-butyl group signals [δ_H 4.12 (2H, t, J = 6.6, H-1“), 1.62 (2H, m, H-2”), 1.39 (2H, dq, J = 14.8, 7.4, H-3“), 0.93 (3H, t, J = 7.4, H-4”), δ_C 65.9C-1“, 31.9 C-2”, 20.2C-3“, 14.1 C-4”] for compound **1**, which was placed at C-9 based on HMBC correlation from H-1″ [δ_H 4.12 (2H, t, J = 6.6)] to C-9 [δ_C 174.3] (Fig. 2). The *β*-D-configuration of the glucopyranoside was determined by comparison with standard D-glucopyranoside using HPLC method after acid hydrolysis, and the coupling constant shift of the anomeric proton [δ_H 5.44 (d, J = 9.1)] [12]. Thus, compound **1** was elucidated as 3-carboxymethylbutyl ester indole 1-*N*-*β*-D-glucopyranoside, and named as acatechu A accordingly.

Compound **2** was obtained as yellowish powder. The molecular formula of **2** was determined as $C_{22}H_{27}NO_9$ by negative HR-ESI-MS at *m/z* 466.1737 [M + H_2O-H]$^-$ (calcd for $C_{22}H_{27}NO_9^-$, 466.1713). The UV spectrum of **2** showed the absorption maxima at 204 and 285 nm. The IR spectrum implicated the presence of hydroxyl (3375 cm^{-1}), carbonyl (1733 cm^{-1}), and aromatic ring (1513 and 1447 cm^{-1}). The ^{1}H NMR spectrum signals in the region of 7.34–6.58 ppm [δ_H 7.34 (2H, m, H-2″ 6″), 7.31 (2H, d, J = 6.8, H-3″ 5″), 7.27 (1H, t, J = 7.1, H-4“), 6.80 (1H, d, J = 2.7, H-2), 6.69 (1H, d, J = 8.6, H-5), 6.58 (1H, dd, J = 8.6, 2.7, H-6)] indicated the presence of two benzene rings. In addition, the signals of a glucopyranoside unit [δ_H 4.74 (1H, d, J = 7.5, H-1’), 3.90 (1H, dd, J = 12.0, 2.2, H_1-6’), 3.68 (1H, dd, J = 12.0, 5.9, H_2-6’), 3.41 (1H, m, H-3’), 3.42 (1H, m, H-4’), 3.39 (1H, dd, J = 5.9, 2.3, H-2’), 3.36 (1H, d, J = 8.4, H-5’)] were observed in the ^{1}H NMR spectrum of **2**. Two benzene rings signals [δ_C 152.8 C-1, 149.3 C-3, 142.9 C-4, 137.3 C-1”, 130.4C-3″ 5″, 129.9C-2″ 6″, 128.4C-4″, 115.9C-5, 109.9C-6, 103.8C-2], and a glucopyranoside unit signals [δ_C 103.8C-1′, 74.9C-2′, 78.0C-3′, 78.2C-4′, 71.6C-5′, 62.7C-6′] were also observed in the ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of 2,4-dihydroxyphenyl 6-*O*-(3-hydroxy-1-oxo-3-phenyl-propyl)-*β*-D-glucopyranoside [13] suggested that these two compounds were structurally similar. However, two hydrogens in **2** were attached to C-2 and C-9″, instead of two hydroxyl groups in the known compound, respectively. In addition, a methoxy group [3.83 (3H, s)] was attached to C-3 based on HMBC correlations of H_3-OCH_3 [δ_H 3.83 (3H, s)] to C-3 [δ_C 149.3] (Fig. 2), and an amino-group attached to C-8“ based on downfield shift of C-8” [δ_H 3.78 (1H, dd, J = 8.9, 4.3), δ_C 57.58] [14]. Acid hydrolysis of compound **2** yielded a D-glucose which was identified by comparison with standard D-glucopyranoside using HPLC method. The anomeric proton [δ_H 4.74 (d, J = 7.5)] indicated the β-configuration for the glucopyranosyl moiety [12]. Thus, compound **2** was identified to be 3-methoxy-4-hydroxyphenyl 6-*O*-(2-amino-1-oxo-3-phenyl-propyl)-*β*-D-glucopyranoside, which was named as acatechu B.

In addition, forty-one known compounds including arecoline (**3**) [15], areca (**4**) [15], methyl nicotinate (**5**) [16], ethyl nicotinate (**6**) [16], *β*-D-fructopyranoside, ethyl (**7**) [17], *β*-D-fructopyranoside, butyl (**8**) [17], butyl quinate (**9**) [18], 2,5-dihydro-2-hydroxy-5-oxo-2-furancarboxylic acid (**10**) [19], *β*-D-fructofuranoside, butyl (**11**) [16], resveratrol (**12**) [20], 2-(4-hydroxy-phenyl) ethl-*β*-D-glucopyranoside (**13**) [21], stroside B (**14**) [22], fumalic acid (**15**) [23], *β*-D-glucopyranoside, benzyl (**16**) [24], 5-methyl-2-(1-methylethyl)-cyclohexanol (**17**) [25], 2-hydroxy-5-methoxy-benzoic acid (**18**) [26], 4-hydroxybenzoic acid (**19**) [27], 4-hydroxy-3-methoxy-benzoic acid (**20**) [17], 3-hydroxy-4-methoxy-benzoic acid (**21**) [27], 3,4-dihydroxy-benzoic acid (**22**) [28], 2-hydroxyethl-5-methoxyphenyl-*β*-D-glucopyranoside (**23**) [29], 2-(*β*-D-glucopyranosyloxy)-5-methoxy-benzoic acid (**24**) [30], 3,4-dimethoxyphenyl-*β*-D-glucopyranoside (**25**) [31], 2,4-dimethoxyphenyl-*β*-D-glucopyranoside (**26**) [32], 4-hydroxy-3-methoxyphenyl-*β*-D-glucopyranoside (**27**) [33], (+)-catechin (**28**) [34], (−)-catechin (**29**) [35], liquiritigenin (**30**) [36], quercetin (**31**) [37], cyanidenon (**32**) [28], isorhamnetin (**33**) [38], isorhamnetin 3-*O*-(6”-*O*-*α*-L-rhamnopyransoyl) *β*-D-glucopyranoside (**34**) [39], methyl rhamnoside (**35**) [40], 6-*O*-methylemodin (**36**) [41], chrysophanol (**37**) [41], proanthocyanidin B_1 (**38**) [42], *β*-sitosterol (**39**) [43], stigmastane-3*β*,5,6*α*-triol (**40**) [25], arborinol (**41**) [44], *β*-carotenoid (**42**) [45], and ginseng triol saponin Rg 1 (**43**) [46] were isolated and identified. Their structures were determined by comparing the spectroscopic data with those

reported in literature. The chemical structures and NMR spectra of all known compounds is presented in the Supplementary material.

All isolated compounds were evaluated for their cytotoxic activities against four human cancer cell lines (Hela, BGC-823, Hep G2 and HCT-116). Compounds **26** and **34** showed weak cytotoxicity against BGC-823 cell line with IC_{50} of 15.91 μM and 20.13 μM, respectively.

3. Experimental

3.1. General experimental procedures

The HR-ESI-MS spectra were performed on Waters UHPLC-H-CLASS/XEVO G2-XS Qtof. NMR data were recorded on Bruker AV-600 spectrometers with TMS as an internal standard. Column chromatographic separations were carried out with silica gel, (200–300 mesh and 300–400 mesh), octadecylsilyl (ODS) (Qingdao Marine Chemical Inc., P. R. China) and Sephadex LH-20 (Sigma-Aldrich). Semipreparative HPLC was performed on an Agilent 1206 high performance liquid chromatograph with a Agilent C_{18} (34 mm × 25 cm) column. Fractions were monitored by TLC (Qingdao Marine Chemical Inc., P. R. China) and spots were visualized by heating silica gel plates sprayed with vanillin in 5% H_2SO_4 solution. Microplate reader (EnSpire 2300, PerkinElmer) and CO_2 incubator (New Brunswick) were used in cytotoxicity bioassays.

3.2. Plant material

The dried fruits of *Areca catechu* L. were collected in 2016 from Hainan Province of China. The plant was identified by Prof. Li-min Gong (School of Pharmacy, Hunan University of Chinese Medicine). A voucher specimen (No. 201610) was deposited at TCM and Ethnomedicine Innovation & Development International Laboratory, Innovative Materia Medica Research Institute, Hunan University of Chinese Medicine, Changsha, P. R. China.

3.3. Extraction and isolation

The dried fruits of *A. catechu* (50 Kg) were extracted twice with 1000 L of 95% ethanol for 2 h under reflux extraction. And all the solvents were evaporated under vacuum to obtain crude extract (1.2 Kg). The obtained extract was suspended in water (1500 mL) and partitioned with PE (2 L × 3 times), CH_2Cl_2 (2 L × 3 times), EtOAc (2 L × 3 times) and *n*-BuOH (2 L × 3 times) to get four fractions after removing the solvent under vacuum, respectively.

The PE (197.2 g) and CH_2Cl_2 (7.2 g) fractions were combined and then subjected to silica gel column chromatagraphy (CC) (2000 g, 16 × 35 cm) eluted stepwise with PE-EtOAc system. The collected fractions were combined based on TLC analysis to afford 20 main fractions. These fractions were further separated by silica gel CC using gradient system (PE-EtOAc & CH_2Cl_2-MeOH) to afford **3–6**, **17–21**, and **39–41**.

The EtOAc fraction (34.9 g) was separated by silica gel CC to yield fractions A-E. The subfraction B was purified by sillica gel CC (3 × 45 cm; eluted with PE-EtOAc) to afford **12** and **15**. The subfraction D was subjected to column chromatography over sephadex LX-20 (eluting with $CHCl_3$:MeOH = 1:1) to yield **28–33**, and **36– > 38**.

The *n*-butanol fraction (57 g) was separated stepwise on silica gel CC (1500 g, 10 × 32 cm) eluted with $CHCl_3$-MeOH-H_2O system (10:1:0.1, 8:2:0.1, 7:3:0.1, 6:4:0.1, 3:7:0.1, 0:1:0, v:v:v) by increasing polarity, to afford 12 main fractions (Fr. 1 ~ Fr. 12). Fr. 2 (2.6 g) was subjected to silica gel CC (2.5 × 62 cm, 70 g) using gradient system ($CHCl_3$-MeOH-H_2O) to afford **7–11** and thirteen fractions (A_1 ~ A_{13}). Compound **1** (4 mg) was obtained from A_6 by ODS column chromatography (2.5 × 8.5 cm; eluted with MeOH-H_2O). Fr. 5 (600 mg) was purified by sephadex LX-20 CC (2.5 × 96 cm) eluting with $CHCl_3$-MeOH system to yield compound **2** (3 mg) and eleven subfractions (B_1 ~ B_{11}). The subfraction B_6 was purified by ODS column chromatography (2.5 × 8.5 cm; eluted with MeOH-H_2O) to afford **22** and **34–35**. Fr 3 and Fr 4 (2.5 g) were separated by chromatography column on silica gel (2.5 × 52 cm, 80 g) to afford **23–27** and **44–45**. Fr. 6 (2.1 g) was purified by ODS column chromatography (3.5 × 15 cm; eluted with MeOH-H_2O) to yield **16**, **13–14**, and **42–43**.

Table 1
^1H and ^{13}C NMR data (δ in ppm) of **1** (150 and 600 MHz, MeOD).

Position	δ_C	δ_H mult (*J* in Hz)
2	125.4	7.37 (1H, s)
3	109.8	–
3a	129.7	–
4	119.8	7.52 (1H, d, 7.9)
5	120.9	7.09 (1H, m)
6	123.1	7.19 (1H, ddd, 8.2, 7.2, 1.1)
7	111.6	7.54 (1H, d, 8.3)
7a	138.4	–
8	31.9	3.78 (2H, s)
9	174.3	–
1′	86.7	5.44 (1H, d, 9.1)
2′	73.8	3.92 (1H, m)
3′	80.6	3.60 (1H, t, 9.0)
4′	71.5	3.50 (1H, m)
5′	78.9	3.57 (1H, dd, 5.7, 2.2)
6′	62.8	3.87 (1H, d, 2.0)
		3.71 (1H, dd, 12.2, 5.7)
1″	65.9	4.12 (2H, t, 6.6)
2″	31.8	1.62 (2H, m)
3″	20.2	1.39 (2H, dq, 14.8, 7.4)
4″	14.0.	0.93 (3H, t, 7.4)

3.3.1. Acatechu A (1)

Yellowish powder; [α]24.5 D + 0.3 (*c* 0. 2, MeOH); UV (MeOH) λ_{max} (log ε) 206 nm (4.19) and 279 nm (3.42); IR (ART) ν_{max} 3420, 1717, 1576, 1463 cm^{-1}; negative ion HR-ESI-MS *m/z* 416.1673 $[M-Na]^-$ (calcd for $C_{20}H_{27}NO_7^-$, 416.1685); ^1H NMR (600 MHz, MeOD) and ^{13}C NMR (150 MHz, MeOD) data were showed in Table 1.

3.3.2. Acatechu B (2)

Yellowish powder; [α]24 D + 0.15 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 204 nm (4.81) and 285 nm (3.81); IR (ART) ν_{max} 3375, 1733, 1615, 1513, 1447 cm^{-1}; negative ion HR-ESI-MS *m/z* 466.1737 $[M + H_2O-H]^-$ (calcd for $C_{22}H_{27}NO_9^-$, 466.1713); ^1H NMR (600 MHz, MeOD) and ^{13}C NMR (150 MHz, MeOD) data were showed in Table 2.

3.4. Cytotoxicity bioassays

MTT assays were performed to evaluate the cytotoxicity activities against Hela, BGC-823, Hep G 2, and HCT-116 cell lines. Briefly, all cells were seeded in 96-well plates at a density of about 5 × 10^4 cells per well, respectively. Each cancer cell line was exposed to the test compound, which was dissolved in DMSO, at various concentrations for 48 h, with paclitaxel as positive control. After replaced the cell culture with cell medium, 100 μL MTT solution (0.5 mg/mL in DMEM culture medium) was added to each well for additional 4 h incubation. The cell medium containing MTT was then replaced with 150 μL DMSO. Afterwards, the micro-plate was incubated at 37 °C for 5 min and then shaken at room temperature for 15 min for complete dissolution of formazan. Finally, the absorbance of each well at 592 nm was recorded by microplate reader (EnSpire 2300) to determine the relative cell viability [47,48].

In conclusion, two new alkaloids (**1** and **2**), and forty-one known compounds (**3–43**) were isolated from the dried fruit of *A. catechu*. The present study is the first report of phytochemical investigation on the *n*-BuOH of *A. catechu* L. Different from previous reported alkaloids of *A. catechu*, two new alkaloids both were linked with glucopyranoside

Table 2
^{1}H and ^{13}C NMR data (δ in ppm) of **2** (150 and 600 MHz, MeOD).

Position	δ_C	δ_H mult (J in Hz)
1	152.8	–
2	103.8	6.80 (1H, d, 2.7)
3	149.3	–
4	142.9	–
5	115.9	6.69 (1H, d, 8.6)
6	109.9	6.58 (1H, dd, 8.6, 2.7)
1′	103.8	4.74 (1H, d, 7.5),
2′	74.9	3.39 (1H, dd, 5.9, 2.3)
3′	78.0	3.41 (1H, m)
4′	78.2	3.42 (1H, m)
5′	71.6	3.36 (1H, d, 8.4)
6′	62.7	3.90 (1H, dd, 12.0, 2.2) 3.68 (1H, dd, 12.0, 5.9)
1″	137.3	–
2″, 6″	129.9	7.34 (2H, m)
3″, 5″	130.4	7.31 (2H, d, 6.8)
4″	128.4	7.27 (1H, t, 7.1)
7″	174.1	–
8″	57.6	3.78 (1H, dd, 8.9, 4.3)
9	38.3	3.34 (1H, d, 3.8) 3.00 (1H, dd, 14.5, 8.9)
3-OCH_3	56.4	3.83 (3H, s)

moiety. Compound **1**, an indole alkaloid, was isolated from Arecaceae family for the first time. The indole *N*-glycoside derivatives are the regulator of plant growth and development, and analogs of **1** have been isolated or synthesized previously [11,49]. For compound **2**, it is rare that the phenylalanine group attached to C-6′ of glucopyranoside moiety in natural products. In addition, all isolated compounds were evaluated for their cytotoxic activities against four human cancer cell lines (Hela, BGC-823, Hep G2 and HCT-116). Compounds **26** and **33** showed weak cytotoxicity against human gastric cancer cell line BGC-823 with IC_{50} of 15.91 μM and 20.13 μM, respectively.

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Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104276.

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