# Antioxidant and Cytotoxic Phenolic Compounds of Areca Nut(*Areca catechu*)

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**Keywords** *Areca catechu*; Phenylpropanoid; DPPH; Antioxidant activity; Cytotoxic activity

**Article ID** 1005-9040(2010)-01-161-04

## 1 Introduction

*Areca catechu* L.(Palmae), commonly known as an important economical seed crop, is widely cultivated in tropical and subtropical areas, including India, Southeast Asia, East Africa and New Guinea. Areca nut(frequently known as betel nut) is the ripe fruit of the tree *A. catechu*. Areca nut can be chewed and it is a common masticatory in tropical and subtropical countries. It was estimated in the early 1990s that 10% to 20% of the world's population chewed betel quid daily[1]. Areca nut is commonly used in folklore medicine for treatment of various diseases such as dyspepsia, constipation, beriberi and oedema. It's reported that the areca nut of *A. catechu* possesses anthelmintic, anti-inflammatory, antioxidant[2], psychoactive[3,4], antidepressant[5], anti-HIV-1[6] effects and so on. Areca nut contains 50%—60% sugars, 15% lipids (glyceride of lauric, myristic and oleic acid), 15% condensed tannins(phlobatannin, catechin), polyphenolics(NPF-86IA, NPF-86IB, NPF-86IIA and NPF-86IIB)[7] and 0.2%—0.3% alkaloids(arecoline, arecaidine, guvacine and guvacoline)[2]. Although a lot of literature[2,8] showed that areca nut had a strong antioxidant activity, no exact component contributing the antioxidant activity to areca nut has been reported yet. During the course of our searching for antioxidant genus from tropical medicinal plants, 11 phenolic compounds possessing antioxidant activity were isolated by bioassay-guided fractionation from the EtOAc fraction of areca nut, which exhibited considerable antioxidant activity by DPPH assay in our preliminary test. This paper describes the structural determination of these compounds, their antioxidant activities by DPPH assay, and cytotoxic activity by MTT method.

## 2 Material and Methods

### 2.1 General Procedure

All melting points were determined with a Beijing Taike X-5 melting point apparatus and were uncorrected. Optical rotations were measured with a Rudolph Autopol III digital polarimeter. The UV spectra and DPPH radical-scavenging assay were measured and carried out on a Beckman DU800 spectrometer. IR spectra were determined on a Nicolet 380 FTIR spectrometer. The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV-400 NMR spectrometer. The chemical shifts were given on $\delta$ scale with TMS as an internal standard, and coupling constant($J$) was expressed in Hz. The MS data was recorded on API Qstar-Pulsar and VG-Auto-Spec-3000 spectrometers. MTT Radical-scavenging assay was performed on a Microplate reader(EL×800, Bio-Tek Company).

### 2.2 Chemical and Reagents

DPPH and ascorbic acid(Sigma) were used for test antioxidant activity. The cell lines SGC-7901 (human gastric cancer) and SMMC-7721(human liver cancer) were obtained from the Cell Bank of Type Culture Collection of Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Commercial silical gel(Qingdao Haiyang Chemistry Group Co., 200—300 and 60—80 mesh) was used for column chromatography. Precoated silical gel(Qingdao Haiyang Chemistry Group Co.) was used for analytical TLC. Sephadex LH-20(25—100 μm, Merck) was

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Received December 23, 2008; accepted January 13, 2009.

Supported by the National Programs for Science and Technology Development of China(No.2007B127B04).

used for column chromatography.

### 2.3 Plant Material

The areca nut of *A. catechu* was collected from Ding'an County, Hainan Province, China, in March 2006, and identified by associate Professor DAI Zheng-fu of Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen(BL20060306) was deposited.

### 2.4 Extraction and Isolation

The freshly milled areca nut of *A. catechu*(39.7 kg) was exhaustively extracted with 95% EtOH at room temperature. After evaporation, the residue was suspended in water and partitioned with light petroleum, EtOAc and *n*-BuOH successively. The crude extracts of the petroleum, EtOAc and *n*-BuOH fractions showed $SC_{50}$ values($SC_{50}$ value was extrapolated from the liner regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals) of >100, 27.6 and 85.1 μg/mL, by DPPH assay, respectively. The result suggests that the antioxidants were mainly contained in the EtOAc fraction, which further investigation was focused on.

The EtOAc fraction(21.6 g) was subjected to column chromatography over silical gel eluted with $CHCl_3$-MeOH gradients(volume ratio 50:1 to 0:1) to obtain 7 fractions($A_1$—$A_7$). Repeated column chromatography on silica gel chromatography eluted with petroleum ether-acetone gradients(4:1—2:1, volume ratio) and Sephadex LH-20(95% EtOH), led to the isolation of compounds **1**(11.9 mg), **2**( 5.4 mg), **3**(16.6 mg) and **9**(13.6 mg) from $A_1$(2.5 g). Fraction $A_2$(4.3 g) was subjected to Sephadex LH-20 and then subjected to silica gel chromatography to yield compounds **5**(20.0 mg), **6**(19.0 mg), **7**(3.1 mg) and **11**(11.6 mg). Fraction $A_3$(3.4 g) was subjected to Sephadex LH-20 (95% EtOH) to yield compounds **4**(18.2 mg) and **8**(16.1 mg). $A_5$(6.3 g) was subjected to silica gel chromatography eluted with $CHCl_3$-MeOH(20:1—5:1, volume ratio) to yield compound **10**(11.6 mg).

### 2.5 DPPH Radical-scavenging Assay

The DPPH assay was performed as described[9]. In this assay, ascorbic acid was used as positive control, a 0.1 mmol/L solution of DPPH(1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared and to 2 mL of this solution was added 0.1 mL of the antioxidant solution in methanol at different concentrations (1.56—100 μg/mL). The reaction mixtures were incubated at room temperature for 30 min. After incubation, the absorbance was read at 517 nm, and mean value was obtained from three duplicated readings. Scavenging activity was determined by the follow equation: scavenging activity(%) = $[(A_{control} - A_{sample})/A_{control}]\times 100\%$.

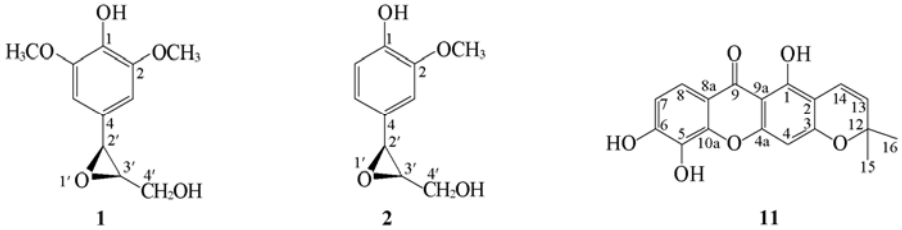
### 2.6 Cytotoxic Activity Assay

The human gastric cancer cell line(SGC-7901) and human liver cancer(SMMC-7721) were maintained in RPMI-1640 supplemented with 10% fetal bovine serum(FBS), 100 units/mL penicillin and 100 μg/mL streptomycin sulfate at 37 ℃, 5% $CO_2$. The MTT assay was performed according to the method described in the previous literature[10].

## 3 Results and Discussion

### 3.1 Identification of Compounds 1—11

Further purification of the EtOAc fraction was carried out with silica gel column chromatograph and Sephadex LH-20. This led to the isolation of 11 phenolic compounds, whose structures were determined to be 4-[3'-(hydroxymethyl)oxiran-2'-yl]-2,6-dimethoxyphenol(**1**), epoxyconiferyl alcohol(**2**), isovanillic acid(**3**), protocatechuic acid(**4**), catalpinic acid(**5**), isorhamnetin(**6**), chrysoeriol(**7**), luteolin(**8**), (±)-4',5-dihydroxy-3',5',7-trimethoxyflavonone(**9**), (2*S*,3*R*)-ent-catechin(**10**) and jacareubin(**11**). Compound **1** was structurally elucidated as a new compound, and compounds **1—9** and **11** were isolated from the genus of *Areca* for the first time. Their structures are shown in Fig.1.




1 2 11

**Fig.1 Structures of compounds 1, 2 and 11**

4-[3'-(Hydroxymethyl)oxiran-2'-yl]-2,6-dimethoxyphenol(**1**), obtained as a light yellow oil, had a molecular formula $C_{11}H_{14}O_5$ based on its HR-ESI-MS (*m/z*=225.0765, calcd. 225.0768 for $C_{11}H_{14}O_5$, $[M-H]^-$), which was supported by its $^{13}C$ NMR and DEPT data. $[\alpha]_D^{26} = -3.0°$(*c* 1.0, $CHCl_3$), UV (MeOH), $\lambda_{max}$/nm: 274. IR(KBr), $\tilde{\nu}_{max}$/cm$^{-1}$: 3419, 1682, 1615, 1516. $^1H$ NMR($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz) data are shown in Table 1.

**Table 1 $^1H$ and $^{13}C$ NMR spectral data of compound 1 in $CDCl_3$**

| Position | $\delta_H$(*J* in Hz) | $\delta_C$ |
|---|---|---|
| 1 | —— | 134.4 |
| 2 | —— | 147.2 |
| 3 | 6.58, s | 102.8 |
| 4 | —— | 132.1 |
| 5 | 6.58, s | 102.8 |
| 6 | —— | 147.2 |
| 2' | 4.73, d, (4.0) | 86.1 |
| 3' | 3.09, m | 54.4 |
| 4'a | 4.28, dd, (8.9, 6.5) | 71.8 |
| 4'b | 3.90, dd, (8.9, 4.0) | —— |
| 2,6-OMe | 3.90, s | 56.4 |

The IR spectrum indicates the presence of hydroxyl(3431 cm$^{-1}$), aromatic(1615 and 1516 cm$^{-1}$) and conjugated carbonyl(1682 cm$^{-1}$) groups. The UV peak at 274 nm also confirms the presence of an oxygenated aromatic ring. The $^{13}C$ NMR data of compound **1**(Table 1) reveals an oxygenated methylene($\delta_C$ 71.8), two oxygenated methines($\delta_C$ 54.4 and 86.1), two methoxyls($\delta_C$ 56.4 and 56.4), and six aromatic carbons of a symmetrical benzene ring($\delta_C$ 147.2, 147.2, 134.4, 132.1, 102.8 and 102.8). The $^1H$ NMR spectrum of compound **1** shows two singlet aromatic protons at $\delta_H$ 6.58(2H, s), two OMe at $\delta_H$ 3.90(6H, s), and one phenolic OH at $\delta_H$ 5.52. Oxirane protons appears as a multiplet at $\delta_H$ 3.09(H3') and a doublet at $\delta_H$ 4.73 (*J*=4.0 Hz, H2'). An observed coupling constant of 4.0 Hz suggests a *cis*-disubstituted epoxide[11]. The remained two signals(dd) at $\delta_H$ 3.90 and 4.28 belong to a methylene group attached to C3'. The position of the substituents at the aromatic ring and the *cis*-configuration of the oxirane protons were confirmed by ROESY experiment(Fig.2). Based on the above evidence, the structrure of compound **1** was elucidated as 4-[3'-(hydroxymethyl)oxiran-2'-yl]-2,6-dimethoxyphenol.

The follow known compounds were identified based on the comparison with data from an extensive literature: epoxyconiferyl alcohol(**2**)[12], isovanillic acid(**3**)[13], protocatechuic acid(**4**)[14], catalpinic acid (**5**)[15], isorhamnetin(**6**)[16], chrysoeriol(**7**)[17], luteolin (**8**)[18], (±)-4',5-dihydroxy-3',5',7-trimethoxyflavonone (**9**) and the optical rotation($[\alpha]_D^{26}$=0°) indicates that compound **9** was isomers with (±)-4',5-dihydroxy-3',5',7-trimethoxyflavonone[19], (2*S*,3*R*)-entcatechin (**10**)[20] and jacareubin(**11**)[21].

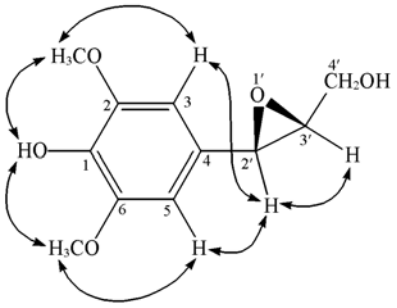



**Fig.2 Key ROESY correlations of compound 1**

### 3.2 Antioxidant Activity

DPPH(1,1-diphenyl-2-picrylhydrazyl) radical-scavenging assay is widely used to evaluate antioxidant capacity in a short time[22]. The antioxidant activities of compounds **1—11** isolated from areca nut were determined by DPPH radical-scavenging assay and the results are shown in Table 2. Most of the isolated compounds exhibit considerable scavenging activity on DPPH assay.

**Table 2 Antioxidant activity of compounds from areca nut on DPPH assay***

| Compound | $SC_{50}$/(μmol·L$^{-1}$) |
|---|---|
| **1** | 41.8 |
| **2** | 81.7 |
| **3** | 124.9 |
| **4** | 73.4 |
| **5** | 182.4 |
| **6** | 146.6 |
| **7** | 172.2 |
| **8** | 32.8 |
| **9** | 255.7 |
| **10** | 19.2 |
| **11** | 19.7 |
| Ascorbic acid(positive control) | 28.9 |

* $SC_{50}$: radical-scavening activity(concentration in μmol/L required for 50% reduction of DPPH radical).

(2*S*,3*R*)-Ent-catechin(**10**) and jacareubin(**11**) exhibit a stronger scavenging activity than ascorbic acid($SC_{50}$=28.9 μmol/L). Catechin and its analogues are varied and distributed widely. They have a significant bioactivity on clearing free radicals, anti-cancer, anti-flammatory, anti-allergic, anti-mutation, improving liver function, and so on[23]. In food industry,

catechin and its analogues can prevent the oxidation and degeneration of lipid and prolong the storage time[24]. Jacareubin was reported to have anti-inflammatory[25] and $H^+$, $K^+$-ATPase activity[26]. And this is the first report about the antioxidant activity of jacareubin.

Compounds **4** and **8** show quite higher antioxidant activities than their analogues, compounds **3**, **5**, **6**, and **7**, due to their possessing an *o*-dihydroxy phenyl structure, which conferred higher stability in the radical from and participating in electron delocalization. This conclusion is consistent with those reported in the literature[27].

Compound **9,** a flavonone, gives a weak activity($SC_{50}$=255.7 μmol/L) due to the lack of conjugation provided by the C2—C3 double bond with the 4-oxo group[27]. While the new compound **1** and its analogue (**2**) give a considerable antioxidant activity.

### 3.3 Cytotoxic Activity

Compounds **1—11** were evaluated for their cytotoxic activity against human gastric cancer cell line(SGC-7901) and human liver cancer(SMMC-7721) by means of the MTT method. Compound **11** shows a significant cytotoxic activity against the SGC-7901 and SMMC-7721 cell lines with the $IC_{50}$ values of 5.1 and 9.3 μg/mL, while the other compounds were inactive(>100 μg/mL).

## 4 Conclusions

In this study, a new phenylpropanoid and 10 known phenolic compounds were isolated from the EtOAc extract of areca nut. All but catechin were isolated from the genus of *Areca* for the first time. All of them showed a degree of antioxidant activity, while only jacareubin possesses a strong cytotoxic activity. The above results will provide the evidence to evaluate the biological functions of areca nut and promote the reasonable usage of it.

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