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Isolation of catechins from *Cycas armstrongii* Miq. of an Egyptian origin

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ABSTRACT

The phytochemical investigation of *Cycas armstrongii* Miq. leaves and twigs using different chromatographic techniques led to the isolation of three catechin monomers; catechin **(1)**, epicatechin **(2)**, and epigallocatechin-3-gallate **(3)**, for the first time from this species. The chemotaxonomic significance of the isolated compounds is discussed in the rest of this article.

INTRODUCTION

The genus *Cycas* is the only genus in the Cycadaceae family; it is considered as the primary progeny of Cycadophyta or the living cycads (Lindstrom *et al.*, 2007). *Cycas* plants belongs to the order Cycadales, which includes 11 genera of both tropical and subtropical plants which generate terminal oblong cones containing seeds. The most cultivated species is *Cycas revoluta* Thunb., which is a slow-growing plant that reaches 2–5 m in height.

Cycas is represented in Egypt by nine species: *Cycas armstrongii* Miq., *C. revoluta* Thunb., *Cycas circinalis* L., *Cycas rumphii* Miq., *Cycas thouarsii* R.Br., *Cycas pectinata* Griff., *Cycas tansachana* K.D. Hill, *Cycas litoralis* K.D. Hill, and *Cycas media* R.Br (Ismail *et al.*, 2020).

In a recent publication (Ismail *et al.*, 2020), we announced the isolation and identification of various components for the first time from this species belonging to different classes: flavonoids, phenolic acids, and sterols. A former phytochemical study of other *Cycas* species constituents, such as *C. revoluta* Thunb. and *C. circinalis* L., resulted in the isolation of 15 biflavonoids, two dihydrobiflavone glycosides, six flavonoid glycosides, four flavan-3-ols, one flavanone, two norisoprenoids, and three lignans (Moawad *et al.*, 2010, 2014).

The aim of this study is to make an intensive phytochemical study of the leaflets of the plant under investigation and isolation of the secondary metabolites using various chromatographic techniques and their structure elucidation by spectral means.

MATERIALS AND METHODS

Identification and extraction

This study deals with *C. armstrongii* Miq., which was obtained from the garden of Zoheria, Giza, Egypt, identified by Dr. M. Abd-Elhaleem (Plant Taxonomy Department, Agricultural Research Center, Cairo, Egypt). Sample no. BuPD41 was kept at the Pharmacognosy Department, School of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

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Shade-dried and powdered leaves of *C. armstrongii* Miq. (A, 1,200 g) were exhaustively extracted, at room temperature, by maceration with 80% methanol (5L X 3). The solvent, under reduced pressure, was evaporated at 40°C yielding 152 g of dried total extract, of which 150 g was suspended in water (250 ml), successively fractionated by solvents of increasing polarity: *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol.

Chromatographic separation

Column chromatography of 1.25 g of the remaining mother liquor was carried out using 50 g spherical regular silica (Sorbtech, Norcross GA) and isocratically eluted with 65% DCM/MeOH, followed by Reversed phase HPLC (RP-HPLC) using Phenomenex column, 250*10 mm Luna 5uC18-7774347-1 with (H_2O and 0.05% formic acid; (A) and (MeOH and 0.05% formic acid; (B) in 65/35 A/B with 10 ml/minutes flow rate, at 270 nm UV detection, affording the following three compounds (Fig. 2): compound **1** (t_R = 7.1 minutes, 15 mg), compound **2** (t_R = 8.5 minutes, 12 mg), and compound **3** (t_R =13.3 minutes, 7.5 mg).

RESULTS AND DISCUSSION

Structure elucidation of the isolated compounds (Table 1 and Figs. 1, 3–11) was achieved by a detailed analysis of their mass and Nuclear magnetic resonance spectroscopic (NMR) spectroscopic data (see Supplementary Data).

Catechins epimers (**1**, **2**) have the same molecular formula, which was determined as $C_{15}H_{14}O_6$ and showed 15 carbon signals in their ^{13}C NMR spectrum. The ^{1}H NMR spectrum could explain the similarity and at the same time the difference in ring C. The magnitude of the coupling between H-2 at δ_H 4.47 (d, J = 7.5) and H-3 at δ_H 3.83 (dd, J = 13.3, 7.5) in compound **1** confirmed the

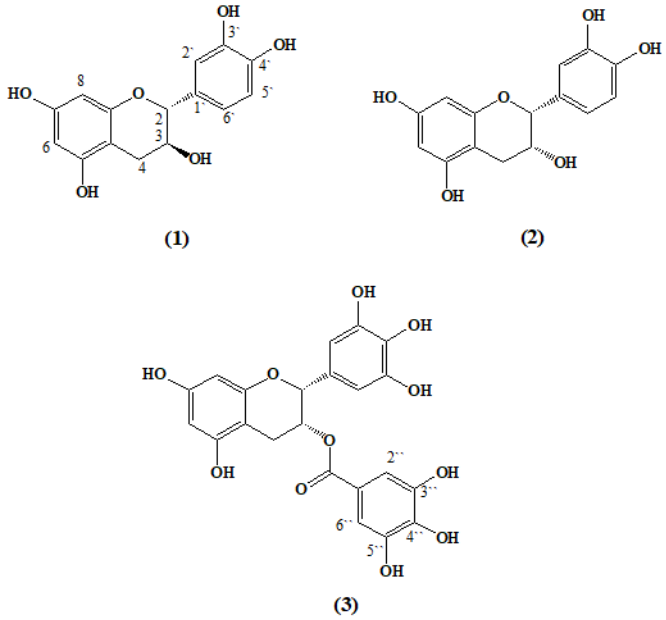

Figure 1. Chemical structure of compounds **1–3.**

Table 1. ^{1}HNMR, ^{13}CNMR data of compounds **(1–3)** in DMSO-d_6 (500 MHz/126 MHz).

	1			2		3
Position	**δC**	**δH (*J* in Hz)**	**Δc**	**δH (*J* in Hz)**	**δC**	**Δh (*J* in Hz)**
2	81.4	4.48 (d, *J* = 7.5 Hz)	78.5	4.74 s	76.9	4.97 s
3	66.8	3.82 (q, *J* = 7.2 Hz)	65.4	4.01 s	68.5	5.37 s
4	28.2	2.35 (dd, *J* = 16.0, 8.1 Hz) 2.66 (dd, *J* = 16.0, 5.4 Hz)	28.2	2.47 (d, *J* = 3.8 Hz) 2.70 (dd, *J* = 16.3, 4.4 Hz)	26.2	2.67 (d, *J* = 16.4 Hz) 2.94 (dd, *J* = 17.1, 4.4 Hz)
5	156.6		156.7		156.1	
6	95.6	5.69 s	95.5	5.72 s	96.0	5.84 s
7	156.9		157.0		157.0	
8	94.4	5.89 s	94.5	5.89 s	94.8	5.95 s
9	155.8		156.2		156.1	
10	99.6		98.9		97.8	
1′	131.1		131.1		132.8	
2′	114.9	6.72 (d, *J* = 2.1 Hz)	115.2	6.89 s	105.9	6.42 s
3′	145.3		144.9		145.9	
4′	145.3		145.0		129.1	
5′	115.6	6.69 (d, *J* = 8.0 Hz)	115.4	6.67 (dd, *J* = 16.3, 4.4 Hz)	145.9	
6′	118.9	6.60 (dd, *J* = 8.2, 2.0 Hz)	118.4	6.67 (dd, *J* = 16.3, 4.4 Hz)	105.9	6.42 s
1′′					119.7	
2′′					109.1	6.83 s
3′′					146.1	
4′′					139.0	
5′′					146.1	
6′′					109.1	6.83 s
Carbonyl					165.7	

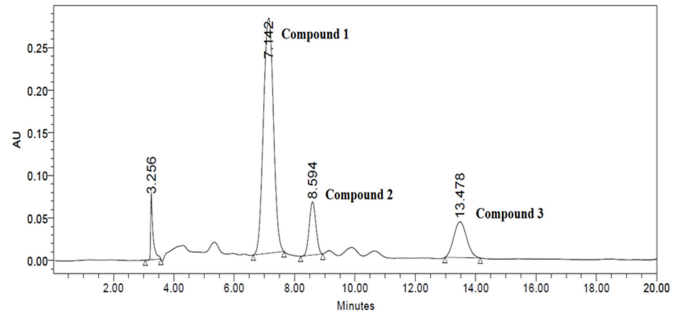

Figure 2. HPLC chromatogram of compounds 1–3.

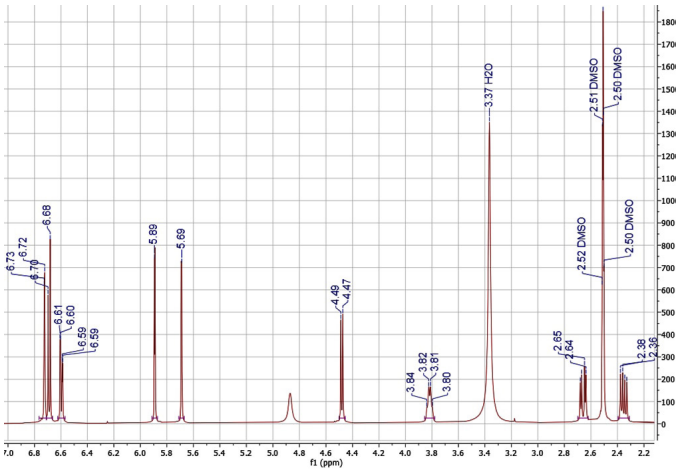

Figure 3. [1]H NMR data of compound **(1)** in DMSO-*d*6 using 500 MHz.

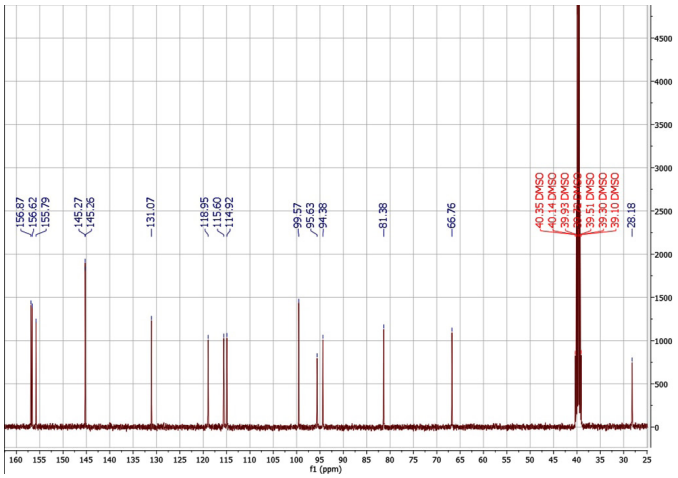

Figure 4. [13]C NMR data of compound **(1)** in DMSO-*d*6 using 500 MHz.

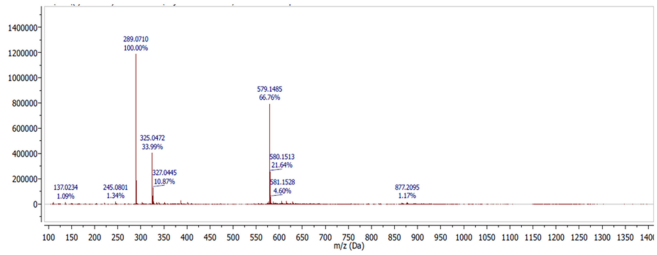

Figure 5. HR-ESIMS data of compound **(1)**.

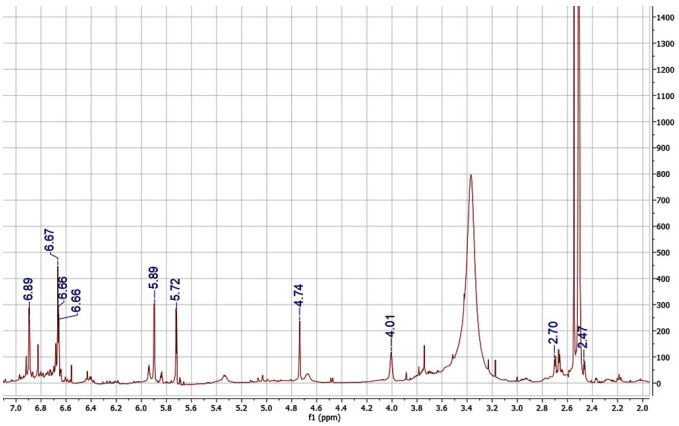

Figure 6. [1]H NMR data of compound **(2)** in DMSO-*d*6 using 500MHz.

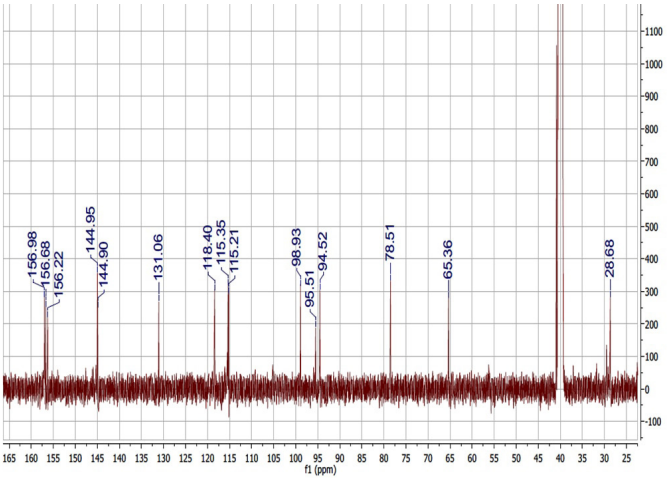

Figure 7. [13]C NMR data of compound **(2)** in DMSO-*d*6 using 500 MHz.

trans stereochemistry between these protons, while in compound **2** the appearance of H-2 and H-3 as a singlet at δH 4.74 (br s) and 4.01 (s) explained the *cis* configuration, which was further supported by the comparison with the literature values (Moawad *et al.*, 2010; Yang *et al.*, 2003). The isolated compounds were identified as catechin **(1)**, epicatechin **(2)**, and epigallocatechin-3-gallate **(3)**.

Flavonoids and polyphenols mark the family Cycadaceae as the main chemical constituents (Moawad, 2011). Catechins epimers (**1**, **2**) were previously obtained from *C. circinalis* (Moawad *et al.*, 2010). Compound **(3)** was isolated from *Myrica rubra* (Yang *et al.*, 2003) and herby is new from the genus *Cycas* and first reported from the family Cycadaceae.

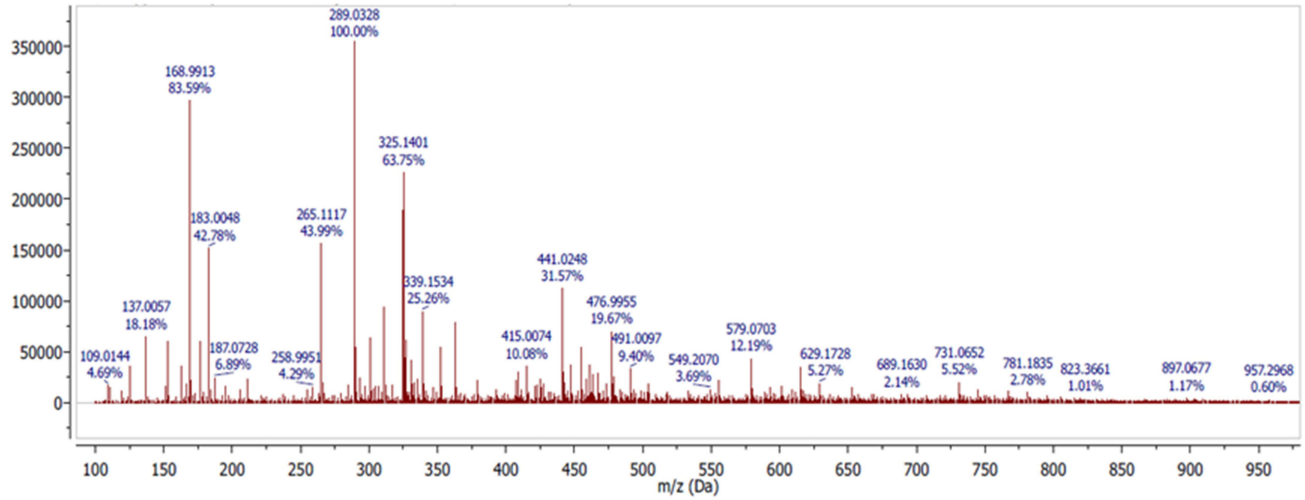

Figure 8. HR-ESIMS data of compound **(2)**.

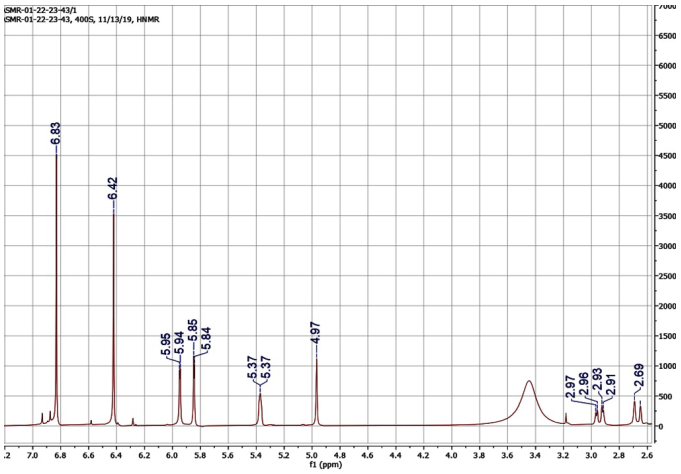

Figure 9. [1]H NMR data of compound **(3)** in DMSO-*d*6 using 500 MHz

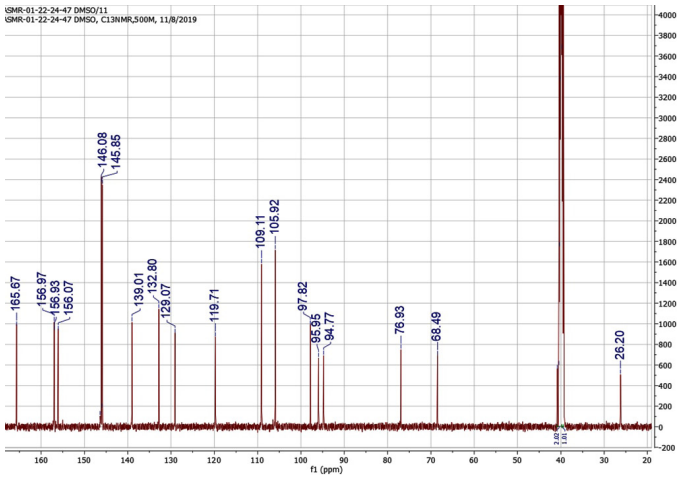

Figure 10. [13]C NMR data of compound **(3)** in DMSO-*d*6 using 500 MHz.

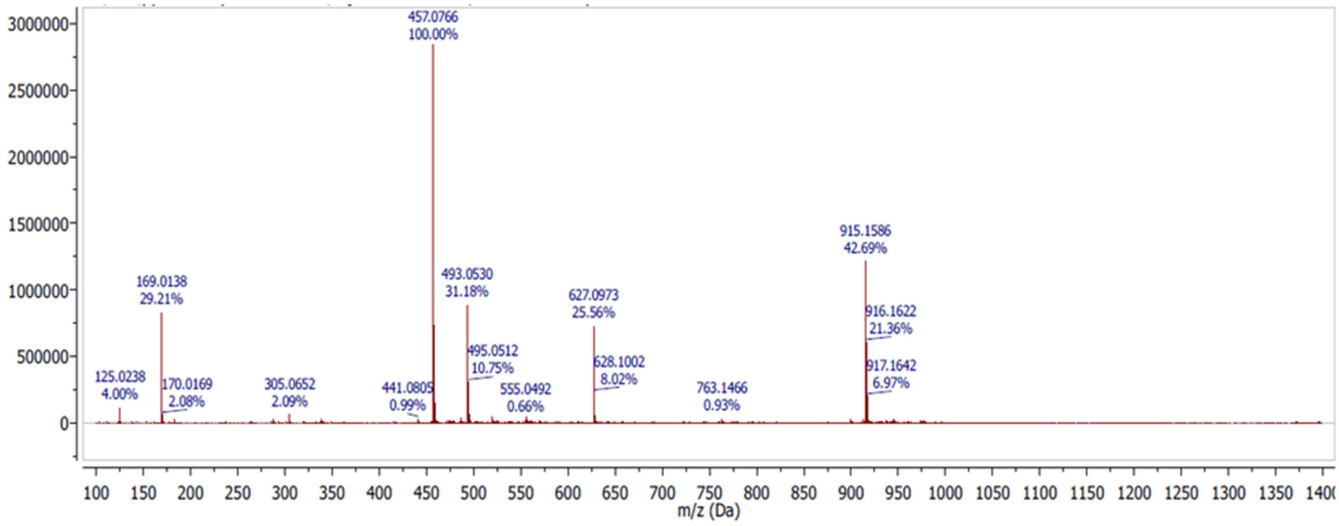

Figure 11. HR-ESIMS data of compound **(3)**.

CONCLUSION

The isolated compounds from *C. armstrongii* Miq. are identified as catechins (**1**–**3**), which are first reported from the plant under investigation, and underscored the taxonomic position of *C. armstrongii* Miq. under the family Cycadaceae with regard to chemotaxonomy. Therefore, catechins can represent the chemosystematic markers of the genus *Cycas*.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

ETHICAL APPROVAL

This study does not involve the use of animals or human subjects.

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