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Melanogenesis inhibitors from *Coix lacryma-jobi* seeds in B16-F10 melanoma cells

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ABSTRACT

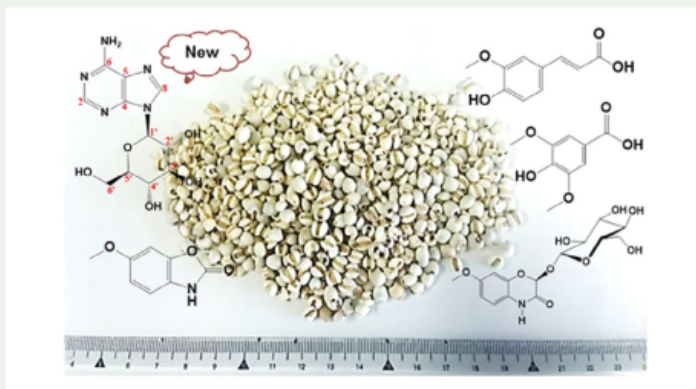
An adenine derivative, 9- β -D-glucopyranosyl adenine, reported for the first time from a natural source, in addition to nine known compounds were isolated from the seeds of *Coix lacryma-jobi*. Their structures were elucidated based on extensive spectroscopic and chemical studies. The isolated compounds and the ethanol extract have been assayed for melanin inhibition using B16-F10 melanoma cell line. The results of our study suggested the potential use of *Coix lacryma-jobi* seeds as a skin whitening agent and reveal the seeds to be a rich source of important phytochemicals with melanogenesis inhibitory activity. Among the isolated compounds, coixol (2) and 2-O- β -glucopyranosyl-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (8) exhibited potent melanogenesis inhibitory activity with no obvious melanocytotoxicity. The rest of the compounds showed weak to moderate activity.

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1. Introduction

Adlay (*Coix lacryma-jobi*, Poaceae) or Job's tears is a grass crop, native to tropical Asia from India to peninsular Malaysia. It has been widely introduced elsewhere and has become naturalised throughout the tropics and subtropics in Africa, the southern United States and the New World tropics (Lim 2013). The seeds are known as Chinese pearl barley and soft-shelled Job's tears. The seeds have long been used in traditional Chinese medicine (TCM) to treat inflammation, dysfunction of the endocrine system also for the treatment of warts, chapped skin, rheumatism and neuralgia, and also as a diuretic, antitumor and analgesic agent besides being a nutritious food (Chung et al. 2011). Numerous recent reports have indicated the beneficial effects of adlay seeds to the human body (Lee et al. 2008; Wang et al. 2016). In an effort to find a new whitening agent, we examined *Coix lacryma-jobi* seeds and their isolated compounds with the aim of developing effective treatments against hyperpigmentation. Hyperpigmentation is a harmless skin condition in which patches of skin become darker in colour. This darkening occurs when an excess of melanin deposits in the skin. Common forms of hyperpigmentation are melasma, lentigo and age spots, which are associated with ageing and exposure to solar ultraviolet (UV) radiation in addition to the hormonal changes and the free radicals generated by UV radiation and others. Therefore, melanocytes are stimulated in a defensive way to protect the skin through overproduction of melanin which is then distributed in the skin epidermis unevenly, leading to dark skin and hyperpigmentation (Briganti et al. 2003; Lim et al. 2009; Yamasaki et al. 2015). We have been searching for inhibitors of melanin production from natural medicines to develop skin whitening agents. Our study is the first of its kind to evaluate the ability of *Coix lacryma-jobi* seeds and its isolated compounds to inhibit melanogenesis in B16-F10 melanoma cell line.

2. Results and discussion

Chemical studies of the different fractions of adlay using different chromatographic techniques afforded 10 compounds (1–10) (Figure 1), of which 9 were identified as β -sitosterol (1), coixol (6-methoxybenzoxazolinone) (2), vanillic acid (3), syringic acid (4), syringaldehyde (5), *trans p*-coumaric acid (6), ferulic acid (7), 2-O- β -glucopyranosyl-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (8) and adenosine (9).

Compound 10 was obtained as a white amorphous powder. It gives positive Molisch's test indicating its glycosidic nature. HR-ESI-MS spectrum gave a molecular ion peak at m/z 297.1119 $[M]^+$, in accordance with the molecular formula $C_{11}H_{15}N_5O_5$. Its UV spectrum (MeOH) exhibited strong absorption peaks at 206 and 259 nm which suggested a purine nucleoside (Sangster & Stuart 1965). This was further supported by the spectral data as follows: ^{13}C NMR spectral data revealed the presence of 11 signals. The signals at δ_c 104.2 (C-1'), 75.5 (C-2'), 81.1 (C-3'), 71.7 (C-4'), 78.0 (C-5') and δ_c 62.8 (C-6'), respectively, could be assigned to a hexopyranosyl moiety. The hexopyranoside shows the ^1H and ^{13}C NMR similar to those of D-glucose (Baumeler et al. 2000) and this was confirmed through acid hydrolysis of the compound and co-chromatography with authentic sugars. The anomeric configuration was assigned as β as a result of the large coupling constant of the anomeric proton [δ_H 4.39 (d, $J = 7.8$ Hz)]. Based on the previous finding, the glycone moiety was identified as β -D-glucose. The other five carbon signals, revealed by the ^{13}C NMR spectrum, could be assigned to a 6-amino-purinylyl moiety (adenine) as follows: ^1H NMR spectrum revealed two singlet peaks

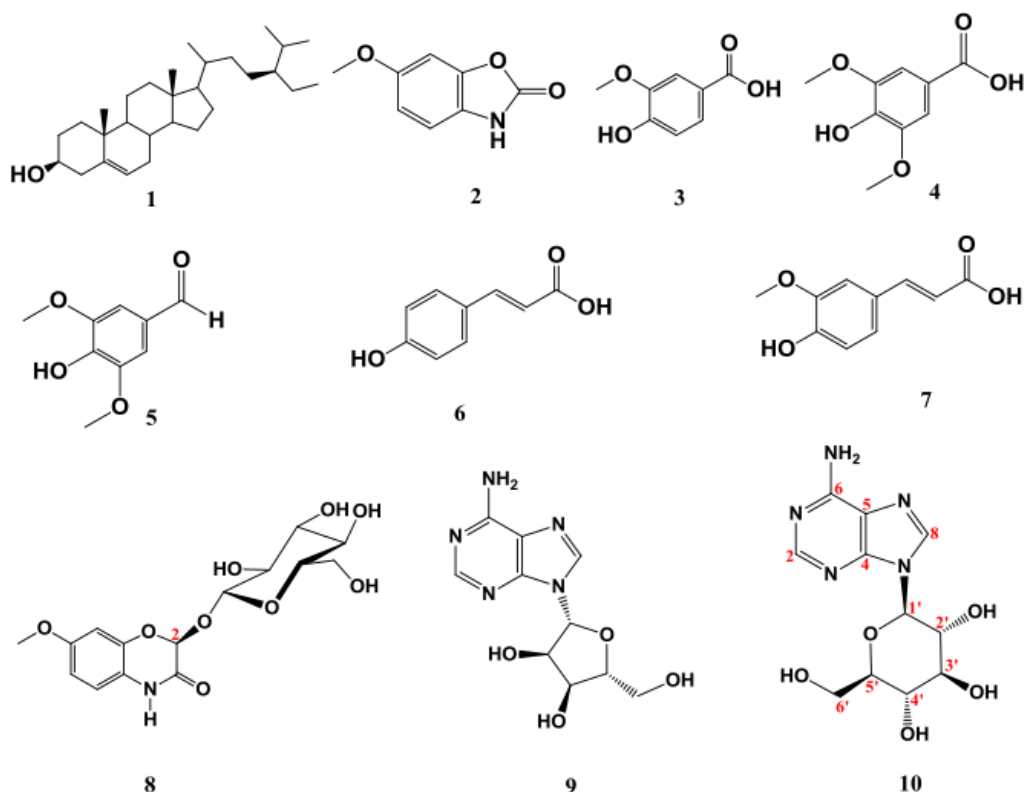


Figure 1. Structures of the isolated compounds (1–10).

at δ_{H} 8.18 and δ_{H} 8.11, correlated to δ_{C} 153.7 and δ_{C} 141.3, respectively, through HSQC spectrum. These two singlet peaks at δ_{H} 8.18 and δ_{H} 8.11 could be assigned to H-2 and H-8 of the 6-amino-purinyl moiety. Also the ^{13}C NMR spectrum revealed the presence of three quaternary carbons at δ_{C} 149.0, 126.4 and δ_{C} 161.5 which were assigned to C-4, C-5 and C-6, respectively, of the adenine moiety. The adenine moiety was further confirmed through co-chromatography with authentic adenine sample. The sugar linkage to the adenine moiety was established from the HMBC cross-peak correlating the β -D-glucose anomeric proton at δ_{H} 4.39 (d, $J = 7.8$ Hz) with the carbon signal at δ_{C} 141.3 (C-8) confirming that the sugar moiety is linked to N-9. IR spectrum of compound **10** showed intense absorption broad bands at 3000–3500 cm^{-1} for hydroxyl and amino groups' stretching. The broad nature of the hydroxyl group stretching usually masks the bi-forked peaks associated with the amino group. It also showed peaks around 3140 cm^{-1} (=CH stretching), 2936 cm^{-1} (CH stretching), 1679 cm^{-1} (C = N stretching), 1603 cm^{-1} (overlap N–H bending, C = C stretching) and 1102 cm^{-1} (C–O stretching) (Silverstein et al. 2005). From the previous findings, the compound was identified as 9- β -D-glucopyranosyl adenine. To the best of our knowledge, this compound is reported here for the first time from a natural source, but it has been previously synthetically prepared (Onodera & Fukumi 1963; Onodera et al. 1966; Patching et al. 2005).

The structures of the known compounds were identified by comparing their physico-chemical and spectroscopic data with those reported in the literature as β -sitosterol (**1**), coixol (6-methoxybenzoxazolinone) (**2**) (Giang et al. 2009), vanillic acid (**3**) (Sakushima et al. 1995; Kim et al. 2006), syringic acid (**4**) (Liao et al. 2014), syringaldehyde (**5**) (Chiji et al. 1980),

trans *p*-coumaric acid (**6**) (Takahashi et al. 1999; Liao et al. 2014), ferulic acid (**7**) (Liao et al. 2014), 2-*O*- β -glucopyranosyl-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**8**) (Nagao et al. 1985) and adenosine (**9**) (Kun et al. 1991). Some of the isolated compounds have been reported before in *Coix lacryma-jobi* seeds; compound (**1**) has been reported by Wu et al. (2007), compound (**2**) by Chung et al. (2011), compounds (**3**, **6** and **7**) have been recently identified in the seeds by Wang et al. (2016), compound (**4**) by Kuo et al. (2002), compound (**5**) by Chen et al. (2011) and compound (**8**) by Huang et al. (2009). Compound (**9**) is isolated for the first time from *Coix lacryma-jobi* seeds through this study; however, it is reported before in the roots of the same plant (Otsuka et al. 1989; Kim et al. 2016). Compound (**10**) as discussed is a new natural product. The absolute stereochemistry for C-2 in compound (**8**) was identified as being in the *R*-form by the ^{13}C chemical shifts of C-2 and C-1'. According to Kluge et al. (1997), the (2*R*)- and (2*S*)-HMBOA-2-*O*- β -L-glucosides showed remarkable differences in the ^{13}C NMR spectroscopic data of C-2 (*R*: δ_{C} 97.6; *S*: δ_{C} 94.6) and C-1' (*R*: δ_{C} 101.0; *S*: δ_{C} 95.9). Inspired by their work, the ^{13}C chemical shifts of C-1' and C-2 in HMBOA-2-*O*-glycosides were utilised to identify the absolute stereochemistry of C-2 to be the *R*-form by observation of downfield C-2 (δ_{C} 96.7) and C-1' (δ_{C} 104.1) chemical shifts.

After establishing the structures, compounds (**1–10**) were investigated using B16-F10 melanoma cells to evaluate the inhibition of melanogenesis and cell viability at a final concentration of 20 μM using arbutin as a positive control at the same concentration. The effect of the compounds on cell viability refers to the cytotoxic effect of these compounds on melanocytes (melanocytotoxicity). A compound that can inhibit melanogenesis without decreasing the cell viability (i.e. without a cytotoxic effect) would be a good candidate as an antimelanogenic agent. By contrast, a compound that lowers melanin content but causes cytotoxicity is not preferable as an antimelanogenic agent. The results of the assay are shown in (Table 1). Taking into account the cytotoxicity, the most active compounds with potent melanin synthesis inhibition and no obvious cytotoxicity were compounds **2** and **8** (the melanin content in melanoma cells decreased to be about 60%) at a final concentration of 20 μM . The results of inhibition of melanogenesis by these compounds (**2** and **8**) are better than the results obtained with the positive control at the same concentration. Furthermore, these compounds are safe to melanocytes which is reflected by the cell viability, about 98%, using MTT assay. For compounds **4** and **7**, the melanin content in melanoma cells decreased

Table 1. Effects of the isolated compounds and the ethanol extract on melanogenesis and cell proliferation of B16-F10 melanoma cells.

Compound*	Melanin content (MC) %	Cell viability (CV) %
1	77.25 $\pm$ 2.53	82.44 $\pm$ 2.85
2	58.32 $\pm$ 1.44	98.73 $\pm$ 1.17
3	92.41 $\pm$ 2.01	100.04 $\pm$ 0.92
4	68.37 $\pm$ 4.72	100.46 $\pm$ 0.71
5	101.42 $\pm$ 3.91	98.53 $\pm$ 5.12
6	81.81 $\pm$ 2.94	103.20 $\pm$ 4.69
7	69.91 $\pm$ 4.30	99.01 $\pm$ 6.17
8	60.81 $\pm$ 1.93	97.87 $\pm$ 1.69
9	84.02 $\pm$ 2.85	100.56 $\pm$ 3.79
10	81.23 $\pm$ 3.63	99.43 $\pm$ 7.05
Ethanol extract (320 $\mu\text{g/mL}$)	51.27 $\pm$ 4.92	45.53 $\pm$ 2.28
Ethanol extract (160 $\mu\text{g/mL}$)	77.21 $\pm$ 4.23	85.37 $\pm$ 4.63
Positive control	63.31 $\pm$ 2.42	99.15 $\pm$ 5.53

*The results are expressed as mean values $\pm$ SD ($n = 3$). Final concentration of the compounds and the positive control (arbutin) was 20 μM .

to about 70% at a concentration of 20 μ M, reflecting their good activity in inhibition of melanogenesis without affecting the cell viability as shown in (Table 1). Other compounds showed weak to moderate activity. The new compound **10** showed about 20% inhibition of melanogenesis at a final concentration of 20 μ M. All the compounds did not affect the viability of melanocytes, reflecting their safety in inhibition of melanogenesis. The ethanol extract showed about 50% inhibition of melanin at a final concentration of 320 μ g/mL, but with remarkable melanocytotoxicity. By decreasing the concentration to 160 μ g/mL, the extract suppressed the melanin content in the cells moderately without causing obvious cytotoxicity to melanocytes. Our results suggest the potential use of *Coix lacryma-jobi* seeds as a melanogenesis inhibitor and the possible use for it as a skin whitening agent. This is the first investigation to evaluate the ability of *Coix lacryma-jobi* seeds and its isolated compounds to inhibit melanogenesis in B16-F10 melanoma cell line.

3. Conclusion

With the aim of developing effective treatments against hyperpigmentation, we examined *Coix lacryma-jobi* seeds and their isolated compounds for melanin inhibition using B16-F10 melanoma cell line. The data presented reveal the seeds to be a rich source of important phytochemicals that might be skin whitening agents, e.g. coixol (6-methoxybenzoxazolinone) (**2**) and 2-O- β -glucopyranosyl-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (**8**), which exhibited antimelanogenic activity while being safe to melanocytes. The results of inhibition of melanogenesis by these compounds (**2** and **8**) are better than the results obtained with the positive control at the same concentration. These compounds would be good candidates as antimelanogenic agents. Moreover, our study resulted in isolation of a new natural product with weak melanin inhibition at a final concentration of 20 μ M. The inhibition of melanin could be better with increasing concentrations of the compound taking into consideration its safety to the cell line. However, the isolated amount of the new compound is not sufficient enough for further assessment of the activity with higher concentrations.

Supplementary material

Additional supporting information including the experimental section and the UV/IR/1D, 2D-NMR/MS data of the new compound can be found in the online version of this article at the publisher's website.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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