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Short Communication

Chemotaxonomic Significance of the *C*-glycosylflavonoid (Vitexin) in *Pereskia bleo* Kunth. (Cactaceae)

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ABSTRACT

Pereskia bleo (Family; Cactaceae) is a medicinal plant traditionally used by the local people in Malaysia to treat cancer, high-blood pressure, diabetes, rheumatism and inflammation. Generally, the plant leaves are consumed raw or brewed to be taken as decoction. The medicinal effects exhibited by the plant are mainly attributed to various chemical compounds, which can be classified using various approaches such as chemotaxonomy. Besides, the chemotaxonomic approach is also one of the useful methods in the field of plant taxonomy. The aim of this study is to reveal the presence of the *C*-glycosylflavonoids (vitexin) in one of the Cactaceae family member which can be useful to justify its similarities with the Didieraceae family. Isolation and purification of *P. bleo* extracts was conducted using various chromatographic techniques and the compounds were identified mainly through the Nuclear Magnetic Resonance (NMR) analysis particularly. Phytochemical evaluation of *P. bleo* has shown the presence of *β*-sitosterol (1), *β*-sitosterol glucoside (2) and vitexin (3) in the hexane, dichloromethane and ethyl acetate extracts, respectively. The isolation of *β*-sitosterol glucoside and vitexin, a *C*-glycosylflavonoid compound named vitexin, is described for the first time in this species. The occurrence of the *C*-glycosylflavonoids in Cactaceae is of chemotaxonomic importance, showing that Cactaceae (particularly the Pereskiaeae sub-family) and Didieraceae family are similar not only morphologically, but also chemotaxonomically, which contrasts to the previous report.

Keywords: Vitexin, *C*-glycosylflavonoid, *Pereskia bleo*, Cactaceae, Chemotaxonomic.

Introduction

Comparing similarities of constituents present in different organisms is difficult due to the large variability of the compounds. The classification varies in chemotaxonomy involving classical and modern taxonomy.[1] Chemotaxonomic classification is a modern and efficient approach to delineate the species identification based on their chemical constituents; furthermore, the method could contribute to the identification of any relationships between developmental stages.[2,3] This type of classification relies on the chemical similarity between taxa, becoming very useful in searching for new medicinal plants and as a tool for plant taxonomy.

Phenolics are one of the four important group of compounds utilized for the chemotaxonomic classification of plants. They are grouped based on the variation in chemical diversity, distribution and function. The more popular families that have been studied using chemotaxonomy are Malvaceae, Ranunculaceae, Magnoliaceae, Polygonaceae, and Solanaceae.[2] There is yet a lack of chemotaxonomic data on Cactaceae family. Therefore, this study focused on the Cactaceae family using *Pereskia bleo* that had been collected in Malaysia.

They are leafy cacti with substantial leaves and thin stems and are usually referred to as lemon vines, rose cacti or leaf cacti.[4,5,6] According to Santos-Díaz *et al*.,[7] these plants produce a wide range of secondary metabolites involved in defense mechanisms against biotic and abiotic stresses.

Pereskia bleo (Kunth) DC (Cactaceae), commonly known as Jarum Tujuh Bilah (in Malay) and Cak Sing Cam (in Chinese) by the locals, is a plant commonly used by the local communities in Malaysia due to its medicinal properties.[8,9] *Pereskia bleo* is a spiny shrub with distinct orange-red flowers, and is claimed to treat a variety of illnesses including diabetes, hypertension and cancer. It has also been used traditionally in Malaysia for the treatment of diseases associated with rheumatism, inflammation, gastric pain and ulcers, and for revitalizing the body.[8-11] Moreover, its fruit extracts have shown high antioxidant activity due to the presence of carotenoids (lutein).[12] The leaves are generally consumed by the locals either eaten raw as vegetables or brewed as a concoction.[8,9,11] In general, it is believed that by drinking the tea made from the mature leaves (6-7 pieces) everyday, one could prevent and cure the abovementioned diseases.

Due to the fact that there is a lack of chemotaxonomic data on the Cactaceae family as well as a previous report indicated that the *P. bleo* (Cactaceae) is different chemotaxonomically compared to the Didieraceae family, this present study was carried out to identify the presence of the *C*-glycosylflavonoid to justify similarities between the two families.

Materials and Methods

General

The solvents used for the extraction, chromatographic procedures and reactions were analysis grade always, exception to ethanol, which was the commercial grade 96º GL. Activated charcoal (Sigma) was also used to remove chlorophyll (Sandusky *et al*., 2006). The extraction of dried leaves of *P. bleo* was performed using Soxhlet apparatus.

The chromatographic analyses by thin layer chromatography (TLC) were performed using silica gel 60 F254 pre-coated plates (230-400

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Mesh ASTM) (Merck) over aluminum, 20 cm × 20 cm plates on layer thickness 0.25 mm. The eluents were prepared in concentration v/v and the visualization of the compounds was achieved by ultra-violet light (254 nm and 365 nm), followed by spraying with 10% sulphuric acid in ethanol to mark the coloured spots.

The structure elucidation of the isolated compounds was performed by means of the analysis of their spectral, mass spectrum, ^{1}H and ^{13}Carbon Nuclear Magnetic Resonance. The NMR spectra were recorded on a BRUKER TOPSPIN 2.1 NMR System with deuterated chloroform, deuterated methanol or deuterated dimethyl sulfoxide as solvents. Melting points were determined with SMP-1 Stuart Scientific, UK. Optical rotations were measured in a 1 decimeter tube using an Alltech AA-55 polarimeter from Optical Activity Ltd. UV analysis was performed with Cary 300 Scan UV-Visible spectrophotometer and Varian-CaryWinUV program. The FT-IR spectroscopy was recorded in KBr discs on a Perkin-Elmer Spectrum 100 FT-IR Spectrometer (Perkin-Elmer Spectrum).

Plant materials

Fresh leaves samples of *Pereskia bleo* were collected in Kota Bharu, Kelantan, Malaysia in October 2009. The leaves were then left to dry at room temperature prior to grinding process. A specimen of *P. bleo* was identified by Mr. Rafly Syamsir from the Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia, and deposited in its herbarium under the number KL5729.

Extraction and purification of P. bleo extract

Three and a half kilogrammes (3.5 kg) of the dried, ground *P. bleo* leaves were extracted with ethanol using a Soxhlet apparatus with ethanol (approximately 35 L) for 72 hours. Then, the crude ethanol extract of *P. bleo* leaves was treated with activated charcoal overnight to eliminate chlorophyll. The extracts were filtered with Whatman filter paper (24.0 cm) and concentrated using a rotary evaporator. The crude ethanol extract obtained (150.7 g) was submitted to a liquid-liquid partition using hexane, dichloromethane, ethyl acetate and butanol.

The purification of compounds from the hexane extract of *P. bleo* (16 g) was performed by a column chromatography using hexane-ethyl acetate-methanol in gradients of increasing polarity as mobile phase, and silica gel (70-230 mesh, Merck) as stationary phase, yielding 202 fractions. Further column and thin layer chromatography procedures led to the isolation of white crystals (mobile phase: hexane–ethyl acetate, 8:2). This compound was identified as *β*-sitosterol (1). These data were in agreement to the previous report.[8]

Part of the dichloromethane extract of *P. bleo* (19.2 g) was submitted to a chromatographic fractionation using hexane–ethyl acetate–methanol in gradients of increasing polarity as mobile phase, yielding 10 fractions. White precipitates were obtained from fraction number 8. The crystals dissolved in DMSO-d_6 were identified as β-sitosterol glycoside (2).

Furthermore, part of the ethyl acetate extract of *P. bleo* (6.2 g) was submitted to a chromatographic fractionation in a column (silica gel 60 g, 70-230 mesh, Ø column: 3.5 cm) using chloroform–ethyl acetate–methanol in gradients of increasing polarity as mobile phase, yielding 12 fractions. Yellow precipitates were obtained from fraction 5. The crystals dissolved in DMSO-d_6 were identified as vitexin (3).

Results and Discussion

The chemotaxonomic strategy has been successfully used to reveal markers involved in the secondary plant metabolism. Numerous morphological and chemical studies over the past few decades have firmly established the taxonomic position of the Cactaceae family in the Caryophyllales. The genus *Pereskia*, with its wooden stems and succulent leaves, is considered to be the most archaic genus; its subdivisions and taxonomic relationships with the other genera are difficult to establish.[13]

The liquid-liquid partition yielded 53.27 g, 21.46 g, 6.23 g and 65.21 g of hexane, dichloromethane, ethyl acetate and butanol extracts, respectively. Further chromatographic procedures of the hexane extracts led to the isolation of white crystals identified as *β*-sitosterol (1), while *β*-sitosterol glycoside (2) was isolated as white precipitates from the dichloromethane extract, and vitexin (3) was isolated from the ethyl acetate extracts as yellow precipitate.

***β*-sitosterol glucoside (2):** White amorphous powder; R_f: 0.84 (ethyl acetate/methanol 8:2); mp 284-288˚C; IR (KBr) ν_{max}: 3379, 2933, 2867, 1462, 1366, 1255, 926, 800 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6,) δ: 0.99 (*m*, H-1), 1.81 (*m*, H-2), 3.47 (*m*, H-3), 2.36 (*m*, H-4), 5.33 (br, *s*, H-6), 1.96 (*m*, H-7), 1.41 (*m*, H-8), 1.48 (*m*, H-11), 1.13 (*m*, H-12), 1.01 (*m*, H-15), 1.26 (*m*, H-16), 0.66 (*s*, H-18), 0.97 (*s*, H-19), 1.34 (*m*, H-20), 0.91 (*d*, *J*=6.48, H-21), 0.97 (*m*, H-22), 1.16 (*m*, H-23), 0.92 (*m*, H-24), 1.64 (*m*, H-25), 0.81 (*d*, *J*=6.84, H-26), 0.81 (*d*, *J*=6.84, H-27), 1.16 (*m*, H-28), 0.84 (*d*, *J*=7.56, H-29), 4.23 (*d*, *J*=7.8, H-1'), 2.91 (*m*, H-2'), 3.13 (*m*, H-3'), 3.03 (*m*, H-4'), 3.04 (*m*, H-5'), 3.65/3.34 (*m*, H-6'), 4.89 (*d*, 2'-OH, 3'-OH, 4'-OH, *J*=4.68), 4.42 (6'-OH); ^{13}C-NMR (125 MHz, DMSO-d_6,) δ: 36.90 (C-1), 28.69 (C-2), 76.92 (C-3), 38.30 (C-4), 140.42 (C-5), 121.14 (C-6), 29.49 (C-7), 31.39 (C-8), 49.58 (C-9), 36.18 (C-10), 20.70 (C-11), 39.07 (C-12), 41.82 (C-13), 56.15 (C-14), 23.90 (C-15), 27.85 (C-16), 55.41 (C-17), 11.75 (C-18), 19.06 (C-19), 36.18 (C-20), 18.58 (C-21), 35.42 (C-22), 25.60 (C-23), 45.12 (C-24), 28.69 (C-25), 19.67 (C-26), 18.91 (C-27), 22.58 (C-28), 11.63 (C-29), 100.79 (C-1'), 76.92 (C-2'), 73.43 (C-3'), 70.05 (C-4'), 76.74 (C-5'), 61.06 (C-6'); EIMS *m/z* 576 (M)$^{+}$. This is the first report of *β*-sitosterol glucoside in *Pereskia bleo.* NMR data were in fair agreement to that reported by Jayaprakasha *et al*.[24]

Vitexin (3): Yellow precipitate; R_f: 0.76 (ethyl acetate/methanol 8:2); mp 250-252˚C, UV (EtOH) λ_{max} (log ε) 270, 329 nm; IR (KBr) ν_{max}: 3361, 1650, 1611, 1505, 1430 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6,) δ: 3.53-3.85 (6H, *m*, sugar moeity), 4.69 (1H, *d*, *J*=9.8 Hz, H-1''), 6.29 (1H, *s*, H-6 OR H-8), 6.79 (1H, *s*, H-3), 6.90 (2H, *d*, *J*=8.7, H-3', 5'), 8.03 (2H, *d*, *J*=8.7, H-2', 6'), 10.35 (1H, *s*, 4'-OH), 10.84 (1H, *s*, 7-OH), 13.18 (1H, *s*, 5-OH); ^{13}C-NMR (125 MHz, DMSO-d_6,) δ: 164.98 (C-2), 102.54 (C-3), 182.73 (C-4), 155.64 (C-5), 98.12 (C-6), 162.31 (C-7), 104.59 (C-8), 160.28 (C-9), 104.03 (C-10), 121.60 (C-1'), 128.95 (C-2', 6'), 115.79 (C-3', 5'), 161.32 (C-4'), 73.46 (C-1''), 70.82 (C-2''), 78.65 (C-3''), 70.53 (C-4''), 81.83 (C-5''), 61.28 (C-6''); EIMS *m/z*; 432 (M)$^{+}$. This is the first time vitexin is isolated from *Pereskia bleo.* NMR data were in fair agreement to that reported by Zhou *et al*.[3] The 1H and 13C-NMR, and ESI-MS of vitexin are provided in the supplementary material.

Structures of the isolated compounds, *β*-sitosterol (1) (0.81%), *β*-sitosterol glucoside (2) (0.37 %) and vitexin (3) (0.24 %) are shown in Figure 1.

The importance of finding *C*-glucosyl flavonoids in *P. bleo* also lies in the fact that this compound possesses several important pharmacological activities,[14,15] potentially explaining and or justifying a few of the popular uses of this plant. Besides that, the presence or absence of a particular phytochemical in a plant, along with the knowledge of its biochemical synthetic pathways can be used to assign its taxonomic position. Furthermore, the quality and content of the active metabolites in herbs are highly variable, depending on factors such as the species, growth stage during harvesting, geographical origins, climate and cultivation.[3]

The earliest phytochemical study of *Pereskia* sp. was by Doetsch *et al.*[16] who reported the isolation of four alkaloids from *Pereskia bleo*: 3,4-dimethoxy-*β*-phenethylamine, mescaline, 3-methoxytyramine and tyramine. Later, Malek and Wahab[8] isolated four compounds from the ethyl acetate fraction of *P. bleo*: phytol, *β*-sitosterol (1), 2,4-di-tert-butylphenol and vitamin E. Furthermore, on repeated chromatographic purification of the active ethyl acetate fraction, red viscous oil and white coloured needles were obtained and identified as dihydroactinidiolide (red viscous oil) and a mixture of sterols consisting of campesterol, stigmasterol and *β*-sitosterol, other than known compounds like 2,4-di-tert-butylphenol, vitamin E and phytol.[10] According to Salt *et al.*[13], Cactaceae is a predominantly Δ^7-sterol-producing family, and all species in the family also produces Δ^5-sterols. In the present study, compound 1 was previously isolated from ethyl acetate extract of *P. bleo* as reported by Malek and Wahab

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and Malek *et al*.[8,10] However, this is the first study reporting the occurrence of *C*-glucosyl flavonoid (vitexin) in *P. bleo* and in the entire Pereskiaeae subfamily and Cactaceae family.

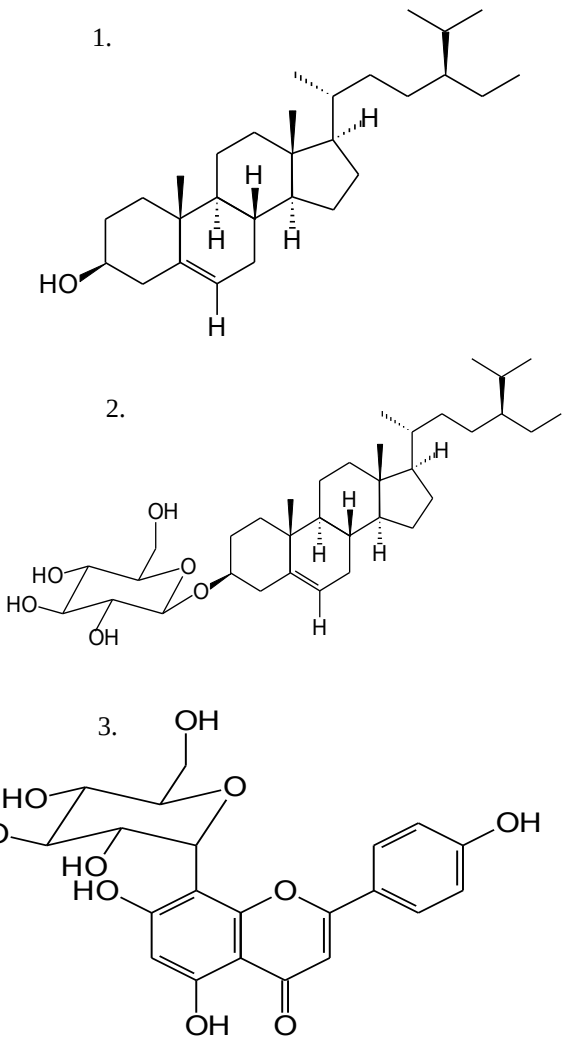

Figure 1: *β*-sitosterol (1), *β*-sitosterol glucoside (2) and vitexin (3), isolated from the hexane, dichloromethane and ethyl acetate extracts of *P. bleo*, respectively.

The *C*-glycosides which possess a direct carbon linkage between sugar and non-sugar are not very prevalent in nature. They are found in only some plants containing anthraquinone derivatives.[2]

C-glycosylflavonoids are predominantly found in Caryophyllales, however, this type of compounds and flavonols are said to be absent in the Cactaceae family. Although Cactaceae is morphologically similar to Didieraceae family, the two families are different chemotaxonomically, according to previous study, by particularly suggesting the Pereskiaeae sub-family to contain kaempferol while the Didieraceae family to contain *C*-glycosylflavones.[17]

In contrast to a previous study by Gupta *et al*.[18] who reported the absence of *C*-glycosylflavonoids in this species, the current study had isolated vitexin from the ethyl acetate extracts of *P. bleo.* Vitexin, a yellow powder, is a *C*-glycosilated flavone with a *D*-glucose linked to the flavonoid skeleton at C-8.[15] The finding is important chemotaxonomically, placing Cactaceae and Didieraceae at similar status, both morphologically and chemotaxonomically. Besides that, Cactaceae also bears morphological similarities with Didieraceae.[17] More specifically, Didieraceae, a family endemic in the Madagascar Island, bears similarity with the taxon Pereskiaeae in Cactaceae.[19] They are economically important as ornamentals.[20]

In addition, the occurrence of *C*-glycosylflavonoids, particularly the vitexin, was also identified in other families such as Tamaricaceae, Plumbaginaceae, Polygonaceae, Droseraceae and Nepenthaceae, which were newly incorporated into the order Caryophyllales.[21]

A survey of leaves and thorns of 22 species of Cactaceae showed the presence of several classes of flavonoids such as flavones (apigenin, baicalein), flavonols (quercetin, kaempferol, isorhamnetin, flavonol-3-methyl-ethers), flavanones (naringenin and its dimethyl-4',7-ether) and flavanonols (taxifolin and aromadendrin). Flavonols are present in Cactaceae family, with kaempferol found in abundance in Pereskiaeae[17,22], such as in spines extract of *P. aculeata*, while *C*-glycosylflavonoids were up to the current study, absent.

Conclusion

In conclusion, compound 3 (vitexin) can be regarded as an important chemotaxonomic marker for the Cactaceae family, showing that Cactaceae (particularly Pereskiaeae) and Didieraceae family are similar not only morphologically, but also chemotaxonomically.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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