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Chemopreventive flavonoids from *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei (Yulangsan) function as Michael reaction acceptors

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ABSTRACT

Natural NQO1 [NAD(P)H quinine oxidoreductase 1] inducing agents play a critical role in cancer chemoprevention. The expression of NQO1 is regulated by Michael reaction acceptors (MRAs) via the Keap1/Nrf2/ARE signaling pathway. The aims of this study were to identify and characterize novel effective chemopreventive agents from naturally occurring products. Using bioassay-guided isolation approaches 16 bioactive MRAs from *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei, also called Yulangsan as a famous Zhuang medicine. The structures were elucidated as chalcone (**1**–**7**), flavonone (**8**–**14**), flavanone (**15**) and isoflavan (**16**). Their electrophilic abilities and NQO1 inducing activity were assessed using GSH (glutathione) rapid screening, and in vitro cell-based (Hepa 1c1c7 cells) assay, respectively. Compounds **3**, **4**, **6**, **13**, and **14** showed to have NQO1 inducing activity. Among them, compounds **4** and **14** interact with NQO1 at Gly 149, Gly 150, Phe 106, Typ 105 and His 161, revealed by molecular docking studies.

Cancer chemoprevention plays an integral role in the overall strategy geared toward controlling the incidence of cancer.[1] It refers to the use of chemical agents that occur naturally in food or administrated as pharmaceuticals to inhibit or reverse the process of carcinogenesis. Epidemiological studies have suggested that consumption of food of plant-origin can decrease the incidence of many cancers.[2] This effect appears to be associated with up-regulation of factors, known as phase II metabolism enzymes.

Phase II metabolism enzymes, including carcinogen and oxidant detoxifying enzymes, quench carcinogens, which are activated by phase I metabolism enzymes, and inhaled endogenous oxidants. The current strategies for effective cancer chemoprevention are to develop agents to modulate the metabolism and disposition of endogenous and environmental carcinogens and oxidants through up-regulation of phase II enzymes.[3,4] NQO1 [NAD(P)H quinine oxidoreductase 1], a phase II metabolism enzyme, can catalyze diverse reactions that collectively result in broad protection against electrophiles and oxidants.[5–7] Many studies have revealed that elevation of NQO1 is correlated with protection against chemical-induced carcinogenesis in animal models.[8,9] Knockout of the NQO1 gene in mice shows significant increases in both carcinogen-induced and spontaneous tumorigenesis.[10–13] The expression of NQO1 is regulated through the Keap1/Nrf2/ARE signaling pathway, which is regulated by Michael reaction acceptors (MRAs). MRAs are a class of active molecules, directly or indirectly involved in various cellular processes in cancer chemoprevention. MRAs contain olefins or acetylenes conjugated to electron-withdrawing functional groups, which confer the ability to conduct the Michael reaction with critical nucleophilic amino acids in electrophile-sensitive proteins, including Keap1.

It has been shown that naturally occurring phytochemicals possess anticancer properties. Among them, flavonoids found in fruits, vegetables, tea, and wine have received considerable attention in recent years.[14,15] They also have high bio-availability in various tissues after being ingested, and, thus, potentially exert their biological effects efficiently.

Recently, we have focused on screening potential natural cancer chemopreventive agents from natural food and herbal medicines, using GSH-conjugation experiment and cell-based (Hepa 1c1c7 cells) bioassays. We have selected a rich source of effective flavonoids, *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei (Yulangsan), out of 40 candidate plants. Then the bioactivity-guided identification, bioassay and molecular docking of effective constituents were carried out successively.

Yulangsan, *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei, belongs to the Leguminosae family. The roots of this plant, also called Yulangsan or Longyanshen, are used as a folk medicine for

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postpartum women and people with certain health conditions, presumably to replenish blood to treat postpartum frail and malnutrition.[16] Modern pharmacological research has revealed that Yulangsan also has properties in cardiovascular protection, antitumor, and hepatoprotective action. The aerial parts of *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei, called Daluosan, is another traditional herbal medicine to eliminate inflammation, alleviate pain, increase blood circulation, and relax and activate tendons in rheumatic arthralgia.[17–19]

NMR spectra were recorded on a Bruker ARX-600 and ARX-400 spectrometer, using TMS as an internal standard. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. of China. HPLC separations were performed on a Hypersil PREP-ODS column (5 μm, 250 × 20 mm) equipped with a Shimadzu SPD-10A UV-detector and a Shimadzu LC-10AT series pumping system (Co., Ltd Japan). The UPLC–DAD analysis was performed on Waters system coupled with a DAD detection, conducting on a acquity H-class BEH-C18 column (1.7 μm, 100 × 2.1 mm) (Waters, America). GSH was produced by Bailingwei Group Co. of China. Specific rotation were recorded on a Polarimeter (Model 341, Perkin Elmer, America).

The roots of *Millettia pulchra* Kurz var-laxior (Dunn) Z.Wei. was collected from Guangxi province of China, in June 2012. The plant material was identified by the professor Jincai Lu. Voucher specimen (no. 20120619) was deposited in the School of Traditional Chinese Material Medica, Shenyang Pharmaceutical University.

The dried roots (4.5 kg) were crushed to small pieces fitting for 10 L flask and reflux extracted with 8 L of 95% ethanol at 100 °C (2 h × 3times) to give a total crude 1040 g. The extract was partitioned into ethyl acetate part (49.5 g) and *n*-butyl alcohol part (55.5 g) successively.[20]

Through the preparative reverse-phase HPLC, fr.1 yielded (*E*)-1-(4-hydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one[21] (**1**) (t_R = 58 min, 1.4 mg), lonchocarpin[22] (**5**) (t_R = 114 min, 8.4 mg) and pongachalconel[23] (**6**) (t_R = 131 min, 1.7 mg) and eluted with 80% methanol. Purpurenone[24] (**7**) (8.5 mg) was isolated from fr.2 through Sephedax LH-20. Pongamol[25] (**2**) (1143.7 mg) and (−) isolonchocarpin[26] (**15**) (9.7 mg) were recrystallizated from methanol and dichloromethane from fr.3. Through column chromatography on silica gel and recrystallizated obtained ovalitenin A[27] (**3**) (86.8 mg) and (*R*)-ovalitenin B[28,29] (**4**) (18.2 mg) from fr.4. Through recrystallizated (PE) obtained karanjin[30](**8**) (3993.2 mg) from fr.5. From fr.7 2″,2″-dimethylchromene-[5″,6″:7,8]-flavone[24] (**11**) (165.5 mg) and lanceolatin B[28] (**9**) (9.6 mg) were obtained by recrystallizated (MeOH) and Sephedax LH-20. From fr.9 [2]benzopyrano[4,3-*b*]-furo[2,3*h*][1]benzopyran-6(8*H*)-one[31] (**10**) (40 mg),

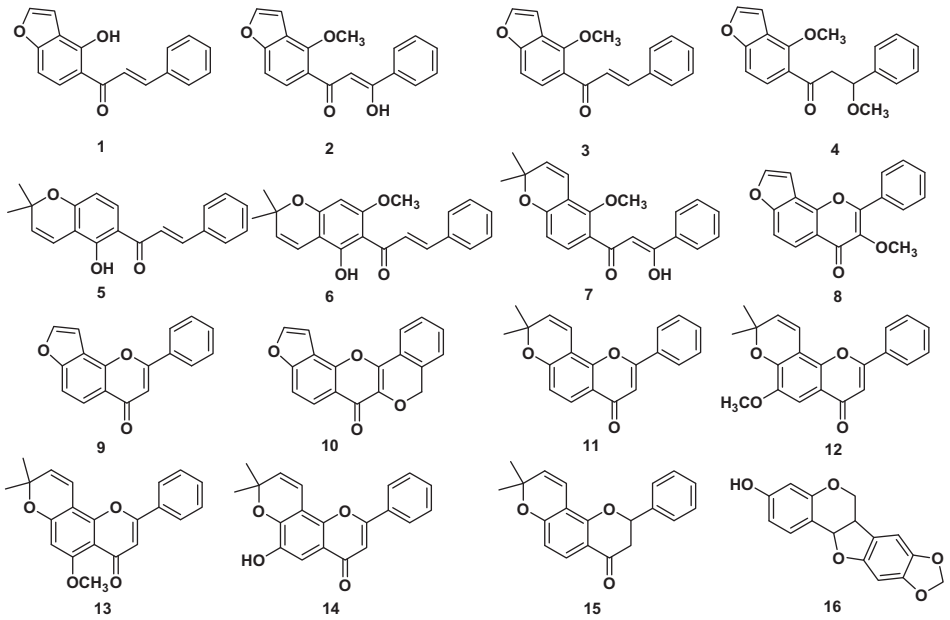

Figure 1. Structures of the isolated compounds of Yulangsan.

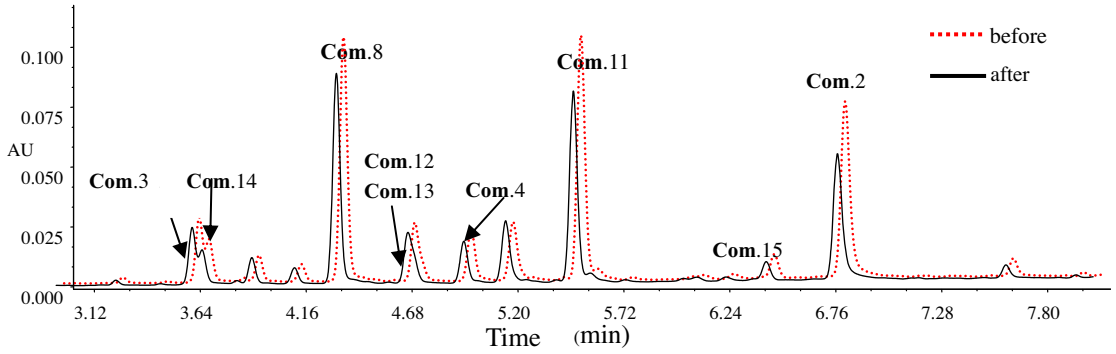

Figure 2. Overlay graphs of UPLC analysis before and after incubation with GSH (recorded at 210 nm).