Antifungal Activity of Pterocarpans from *Caragana jubata* (pall.) Poir.

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ABSTRACT: OBJECTIVE To study the antifungal activity of pterocarpans from *Caragana jubata* (pall.) poir.. **METHODS** The compounds have been separated and purified by polyamine resin and silica gel column chromatography as well as the preparative HPLC method from the active fraction. The minimum inhibitory concentration(MIC) were measured by broth microdilution method according to document M27-A published by the National Committee for Clinical Laboratory Standards (NCCLS). **RESULTS** The $CHCl_3$ extract of the MeOH extract prepared from the whole plant of Caragana jubata (pall.) Poir. exhibited good antifungal activity. Bioassay-directed fractionation led to the purification of five pterocarpans, 3-methoxy maackiain (**1**), maackiain (**2**), 3-methoxy-9-hydroxy pterocarpan (**3**), 3,9-dimethoxy pterocarpan (**4**) and 3-methoxy-4,9-dihydroxy pterocarpan (**5**), in which compounds 2 showed potential antifungal activity against three Candida strains at the level of MICs 6.25-25 μg·mL^{-1}. **CONCLUSION** All of the compounds were isolated from the title plant for the first time. And biological validation showed pterocarpans were responsible for antifungal activity.

KEY WORDS: *Caragana jubata* (pall.) Poir.; Pterocarpans; antifungal activity

鬼箭锦鸡儿中紫檀烷类化合物抗真菌活性研究

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摘要：目的 研究鬼箭锦鸡儿中紫檀烷类化合物的抗真菌活性。**方法** 在生物活性指导下,利用聚酰胺、硅胶常规色谱和制备型高效液相色谱方法进行化合物分离，采用波谱技术和化学方法鉴定化合物的结构。最低抑菌浓度(MIC)测定参照美国国家临床实验室标准化委员会(NCCLS)推荐的 M27-A 方案中的微量肉汤稀释法。**结果** 鬼箭锦鸡儿整株植物氯仿部分显示好的抗真菌活性，活性追踪分离得到五个紫檀烷类化合物，分别是 3-甲氧基高丽槐素(**1**)、高丽槐素(**2**)、3-甲氧基-9 羟基紫檀烷(**3**)、3，9-二甲氧基紫檀烷(**4**)和 3-甲氧基-4，9 二羟基紫檀烷(**5**)，其中化合物 **2** 面对三种念珠菌菌株，显示出潜在的抗真菌活性，最低抑菌浓度范围为 6.25~25 μg·mL^{-1}。**结论** 所有化合物均为首次从该植物分离得到,并且生物活性结果确认紫檀素类化合物是有效的抗真菌成分。

关键词：鬼箭锦鸡儿；紫檀烷；抗真菌活性

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Caragana jubata (pall.) Poir. which is subordinate to Caragana Fabr. in a perennial leguminous bush endemic to the northwest of China. It is one of the oldest medicinal plants used in traditional Tibetan medicines, widely distributed in Qinghai, Tibet, Sichuan, Gansu, Ningxia, Xinjiang and Inner Mongolia provinces, where they live in Shady slope or half-shade slope at an altitude of 3 000-4 700 m[1-3]. The whole plants of *Caragana jubata* (pall.) Poir. have been long used to treat some cardiovascular diseases, such as atherosclerosis, hyperlipidemia, hypertension, blood circulation disorder, blood stasis and so on, and it is also used to treat arthritis and abnormal menstruation, and relieve muscle pain[4-6]. However, the limited chemical principles, resveratrol, Cassigarol E, scirpusin B, a few volatile oil and flavonoids were isolated from *C. jubata* (pall.) Poir[7-8]. As part of our *in vitro* antimicrobial screening efforts, the $CHCl_3$ fraction of the methanol extract from the whole plants of *C. jubata* (pall.) Poir. showed potential antifungal activity against three *Candida* strains, *Candida albicans*, *C. krusei*, and *C. parapsilosis*. Bioassay-guided fractionation led to the purification of five pterocarpan, 3-methoxy maackiain (**1**), maackiain (**2**), 3-methoxy-9-hydroxy pterocarpan (**3**), 3,9-dimethoxy pterocarpan (**4**) and 3-methoxy-4, 9-

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dihydroxy pterocarpan (**5**). All of the compounds were firstly isolated from this plant, and maackiain (**2**) showed potential antifungal activity against three Candida strains at the level of MICs 6.25-25 $\mu g \cdot mL^{-1}$.

1 Instrument and experiment material

1.1 Instruments and regents

DL-CJ-2N Ultraclean working table,MJ-180B Fungi incubators, Electronic Analytical Balance, Abbott Bacterial Analyzer,DK-98-1 Electron constant temperature water bath boiler.

Column chromatography (CC), silica gel (200-300 and 300-400 mesh), polyamide resin (100-200 mesh), Analytical TLC (precoated silica gel plates, GF-254), Preparative and Semi-preparative HPLC system (two PrepStar SD-1 solvent delivery modules, a ProStar UV-Vis 320 detector and a ProStar 701 Fraction Collector), a LiChrospher 100 RP-18 column (220 mm×25 mm, 12 μm), Perkin-Elmer 341 polarimeter, Bruker DRX-400 MHz spectrometer.

Fluconazole and Chloroamphenicol were purchased from Three-dimensional Shanghai pharmacy Ltd.(batch number 20041104 and 20060309), and all other regents were of analytical grade.

1.2 Used microorganisms

1.2.1 Bacterial strains Klebsislla pneumoniae (Kp) was obtained as hospital isolates from Huashan Hospital, Shanghai, Staphylococcus aureus ATCC 25923 (Sa), S. epidermidis ATCC 26069 (Se), Bacillus sublitis ATCC 6633 (Bs), Escherichia coli ATCC 25922 (Ec), Cryptococcus neoformans (Cn) were purchase from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China).

1.2.2 Fungal strains Candida albicans ATCC 64550 (Ca), C. krusei ATCC 6258 (Ck), C. parapsilosis ATCC 22019 (Cp), Torulopsis glabrata (Tg) were purchase from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China).

1.3 Plant material

The whole plants of *Caragana jubata* (pall.) Poir. were collected in September 2006 in Guo-luo county, Qinghai province, PR China and identified by Prof. Jie Duo, Qinghai Institute of Tibetan Medicine. A voucher specimen (No. 2006CJP09) is deposited in the Herbarium of Qinghai Nationalities Institute, Xining, Qinghai province, PR China.

2 Methods and results

2.1 Extraction and isolation

Air-dried whole plants of *Caragana jubata* (pall.) Poir. (1 kg) were ground and then percolated with methanol. The concentrated extract was suspended in H_2O and partitioned successively with hexane, $CHCl_3$, EtOAc and n-BuOH to afford Hexane fraction (34 g), $CHCl_3$ fraction (76 g), EtOAc fraction (45 g), n-BuOH fraction (59 g) and water fraction (31 g), respectively. 20 g of the $CHCl_3$ fraction was subjected to CC over polyamide resin (500 g) eluted with 5 % and 95 % aqueous EtOH. The 95% EtOH fraction (12 g) was subjected silica gel column eluted gradiently with $CHCl_3$/MeOH. 1.3 g of the evaporated residue from $CHCl_3$/MeOH elution was subjected to preparative HPLC (CH_3CN in H_2O from 15 % to 80 %) to yield **1** (10 mg) and **2** (105 mg), respectively. 0.97 g of the evaporated residue from $CHCl_3$/MeOH elution was subjected to preparative HPLC (CH_3CN in H_2O from 25 % to 100 %) to yield **3** (54 mg), yield **4** (5 mg) and yield **5** (13 mg), respectively. The purity of the isolated five compounds was above 95% (HPLC).

2.2 Identification

3-Methoxy maackiain (**1**) colorless crytals (MeOH); mp, 169-169 ℃ ESIMS *m/z* 321 $[M+Na]^+$, 299 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.40 (1H, d, J = 8.6 Hz), 6.72 (1H, s), 6.61 (1H, dd, J = 8.6, 2.6 Hz), 6.42 (1H, d, J = 2.6 Hz), 6.40 (1H, s), 5.90 (1H, brs), 5.85 (1H, brs), 5.46 (1H, d, J = 6.9 Hz), 4.19 (1H, dd, J = 10.9, 4.9 Hz), 3.78 (3H, s), 3.61 (1H, t, J = 10.9 Hz), 3.46 (1H, m). The above data are consistent with those of the litereture's[9-10].

Maackiain (**2**) colorless crytals (MeOH); mp, 179-181 ℃; ESIMS *m/z* 307 $[M+Na]^+$, 285 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.40 (1H, d, J = 8.5 Hz), 6.73 (1H, s), 6.57 (1H, dd, J = 8.5, 2.6 Hz), 6.41 (1H, s), 6.40 (1H, d, J = 2.6 Hz), 5.90 (1H, brs), 5.87 (1H, brs), 5.43 (1H, d, J = 7.0 Hz), 4.18 (1H, dd, J = 11.0, 5.0 Hz), 3.61 (1H, t, J = 11.0 Hz), 3.42 (1H, m). The above data are consistent with those of the litereture's[9-10].

3-Methoxy-9-hydroxy-pterocarpan (**3**) colorless crytals ($CHCl_3$); mp, 64-65 ℃; ESIMS *m/z* 293 $[M+Na]^+$, 271 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.39 (1H, d, J = 8.4 Hz), 7.17 (1H, d, J = 8.8 Hz), 6.57 (1H, dd, J = 8.4, 2.6 Hz), 6.46 (1H, d, J = 8.8, 2.3 Hz), 6.43 (1H, d, J = 2.6 Hz), 6.40 (1H, d, J = 2.3 Hz), 5.46 (1H, d, J = 6.6 Hz), 4.21 (1H, dd, J = 10.4, 4.3 Hz), 3.79 (3H, s), 3.60 (1H, t, J = 10.4 Hz), 3.56 (1H, m). The above data are consistent with those of the

litereture's[11].

3,9-Dimethoxy-pterocarpan (**4**) colorless oil; ESIMS *m/z* 307 $[M+Na]^+$, 285 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.42 (1H, d, *J* = 8.2 Hz), 7.12 (1H, d, *J* = 8.8 Hz), 6.62 (1H, dd, *J* = 8.2, 2.6 Hz), 6.47 (1H, d, *J* = 8.8, 2.3 Hz), 6.45 (1H, d, *J* = 2.6 Hz), 6.42 (1H, d, *J* = 2.3 Hz), 5.50 (1H, d, *J* = 6.6 Hz), 4.21 (1H, dd, *J* = 10.2, 4.4 Hz), 3.78 (3H, s), 3.76 (3H, s), 3.60 (1H, t, *J* = 10.2 Hz), 3.57 (1H, m). The above data are consistent with those of the litereture's[11].

3-Methoxy-4,9-dihydroxy pterocarpan (**5**) colorless crystal; mp, 173～175 ℃; ESIMS *m/z* 309 $[M+Na]^+$, 287 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ7.45 (1H, d, *J* = 8.4 Hz), 6.98 (1H, d, *J* = 8.6 Hz), 6.69 (1H, dd, *J* = 8.4, 2.5 Hz), 6.77 (1H, d, *J* = 8.6 Hz), 6.48 (1H, d, *J* = 2.5 Hz), 5.51 (1H, d, *J* = 6.7 Hz), 4.23 (1H, dd, *J* = 10.2, 4.3 Hz), 3.81 (3H, s), 3.62 (1H, t, *J* = 10.2 Hz), 3.55 (1H, m). The above data are consistent with those of the litereture's[12].

2.3 Experiment Methods

The antimicrobial activity was determined using broth dilution techniques as previously described[13-14].

2.4 Screening for antimicrobial activity

The antimicrobial activity was determined using broth dilution techniques as previously described[13-14]. The solutions (maximum concentration) of the compounds (i.e. the compounds that induced zones of inhibition) were prepared in DMSO, serially (2-fold) diluted and 0.5 mL of each dilution was introduced into a test tube containing 4.4 mL of Selenite broth; then 0.1 mL of bacteria suspension (5×10^5 cfu·mL^{-1}) was added and the mixture was homogenized. The total volume of the mixture was 5 mL, with the test-compound concentrations in the tube ranging from 200 to 6.25 μg·mL^{-1} and those of the standard compounds, i.e. chloroamphenicol and fluconazole, ranging from 256 to 2.0 μg·mL^{-1} and 200 to 1.56 μg·mL^{-1}, respectively. After 24 h of incubation at 37 ℃, the MIC values were reported as the lowest concentration of anti-microbial that prevented visible growth. Results and Compound structural formula as follows Tab 1 and Fig 1.

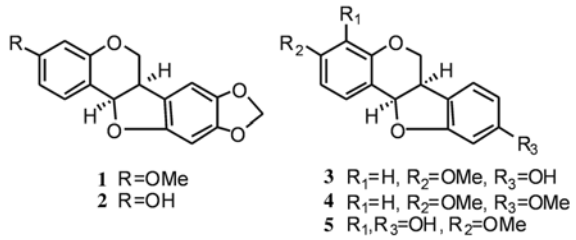
Tab 1 Antimicrobial activity of five fractions and compounds **1-5** in MIC values (μg·mL^{-1})

表 1 5 个组分和 **1**~**5** 化合物的抗菌活性最低抑菌浓度值 (μg·mL^{-1})

	Sa	Se	Es	Ec	Kp	Ca	Ck	Cp	Tg	Cn
Fractions										
Hexane	200	100	>200	>200	200	>200	>200	>200	>200	>200
$CHCl_3$	>200	>200	100	>200	>200	25	50	25	100	>200
EtOAc	>200	200	>200	>200	200	200	>200	200	>200	>200
n-BuOH	>200	200	200	100	200	200	100	200	>200	>200
Water	200	200	>200	100	>200	200	>200	200	>200	>200
Compounds 1	200	200	>200	>200	>200	50	25	25-50	200	100
Compounds 2	200	100	>200	>200	>200	12.5	6.25	25	50	50-100
Compounds 3	>200	>200	200	>200	>200	100	100	50	>200	>200
Compounds 4	>200	>200	>200	>200	>200	50	100	50	>200	200
Compounds 5	>200	>200	>200	>200	>200	25	50	50	200	200
Chloroamphenicol	4.0	4.0	8.0	2.0						
Fluconazole						1.56	50	1.56	6.25-12.5	50

Note: MIC was defined as the lowest concentration that inhibited visible growth. Chloroamphenicol and Fluconazole were used as positive control agents

注：MIC 定义为最低抑菌生长浓度；氯霉素和氟康唑被用于阳性对照药剂



1 R=OMe
2 R=OH

3 R_1=H, R_2=OMe, R_3=OH
4 R_1=H, R_2=OMe, R_3=OMe
5 R_1,R_3=OH, R_2=OMe

Fig 1 Structures of pterocarpans compounds **1-5**

图 1 **1**~**5** 紫檀烷化合物的结构

3 Discussion

The $CHCl_3$-soluble fraction of the MeOH extract prepared from the whole plant of *Caragana jubata* (pall.) Poir. exhibited potentially antifungal activities against three Candida species (C. albicans, C. krusei, C. parapsilosis). The bioassay-guided isolation of the $CHCl_3$-soluble fraction yielded five pterocarpans. The pterocarpan analogues are the major class of constituents of *Caragana jubata* (pall．)Poir．, in which compounds **2** and **3** occurred especially as major second metabolites with high contents in the whole plants. All the isolates were tested on antimicrobial assays, and some of the pterocarpan derivatives described in this work are markedly potent in vitro against three pathogenic fungal strains. The antifungal activity of compounds **1** and **2** against C.

krusei was even more than that of the known antibiotic agent, fluconazole. Antifungal results of compounds **1**–**5** and their structural features have inferred the preliminary patterns of structure-activity relationship. Compounds **1-5** with the pterocarpan skeleton are more or less active, in which compounds **1** and **2** bearing a 8,9-methylenedioxy group are more active than compounds **3-5** with a hydroxyl or methoxy group at position 9 and 10. If the hydroxyl group at position 3 is substituted by the methoxy group, compounds **1**, **3** and **4** becomes less active. Compound **5** with an additional hydroxyl group at position **4** showed stronger activity than **3**.

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