The Ethyl Acetate Extraction Obtained from *Podocarpus nagi* kernel Meal with Anticancer Activity

Yuchen Xiao¹,³,⁴, Jianping Yong²*, Yang Yang¹ and Canzhong Lu¹,²,⁴*

¹Fujian Institute of Research on the Structure of Matter, Haixi Institute, Chinese Academy of Sciences, China.
²Xiamen Institute of Rare-earth Materials, Haixi Institute, Chinese Academy of Sciences, China.
³Shanghai Tech University, China.
⁴University of Chinese Academy of Sciences, China.
* Corresponding Authors E-mail: jpyong@fjirsm.ac.cn

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Cancer is a major public health problem worldwide, and it is one of the top three major diseases in terms of mortality. Some small molecular synthesized drugs have been used clinically. However, much side-effects were also appeared during treatment of the cancer patients with the synthesized anticancer drugs in clinical. Some Chinese Traditional Plant Medicines have ever been used for treatment of cancer with the low side-effects. Thus, it is essential to find anticancer drugs or drug candidates from Chinese Traditional Plant Medicines. Podocarpus nagi contains different kinds of biological components together with a wide spectrum of biological activities, and it has ever been used in the folk of Yao Nationality for treatment different diseases. It is essential to study this folk plant medicine to discover new drugs or drug candidates. In this work, we obtained different polar extractions and evaluated their in vitro anticancer activity.

**Keywords:** Podocarpus nagi kernel meal, Different polar extractions, Anticancer evaluation.
from the Chinese Traditional Plant Medicines have become the mainstream of drug development.

*Podocarpus nagi* (*P. nagi*, named *Zhubai* in Chinese) is widely distributed in south districts of Yangtze River, such as Fujian, Hunan, Guangxi and Guangdong, etc. This plant contains different kinds of biological components (such as volatile oil, flavonoids, steroids, sugar and glycosides, lactones, etc.) and it exhibits a wide spectrum of biological activities: such as hemostasis, bone setting, anti-bacterial, anti-tumor, antiviral, antioxidative and detumescence activities\(^1\). The essential oil of *P. nagi meal* has been reported to exhibit antitumor activity\(^4\). According to the folk records of the Yao Nationality, *P. nagi* has ever been used to treat trauma, stop-bleeding, fractures, knife wounds, gunshot wounds, body odor, eye diseases and colds, etc. The fresh bark or root of *P. nagi* was also used to treat the rheumatoid arthritis \(^5\)\(^-\)\(^6\).

During our previous work, our research group also isolated two new sterols from the leaves of *P. nagi*: 26,27-dinorcholest-5-en-3-\(\alpha\)-ol(1) and (24\(R\))-3\(\alpha\),5\(\alpha\)-dihydroxy-24-ethyl -5\(\alpha\)-cholestan-6-one(2) (Figure 1) and compound 1 exhibited higher anticancer activity against gastric cancer, breast cancer (MCF-7), lung cancer (A549) and Hela cell lines\(^7\). In addition, our research group also prepared the edible oil from the *P. ngai* kernel and this oil exhibited higher antioxidant and anticancer activity\(^8\). In order to find the active new components with anticancer activity from this plant medicine, we studied the *P. ngai* kernel meal (which is the residue after extracting the oil from the *P. ngai* kernel) in current work. We mainly extracted different polar extractions and tested their *in vitro* anticancer activity to select the active extraction to provide the scientific guidance for the below work. The experimental procedure listed in figure 2.

### EXPERIMENT

#### Materials and instruments

The *P. nagi* kernel meal (which is the residue after extracting the oil from the *P. ngai* kernel) was collected in September of 2018 from the Yangli town of Fujian province and powdered; other chemicals used for chemical extractions and biological evaluations are analytical reagents and commercially available.

**Full-wavelength multifunctional microplate reader** (Multiskan GO, USA).

**Detailed processes of extraction**

15 kg powdered *P. ngai* kernel meal was soaked in a solution of \(V_{\text{ethanol}}/V_{\text{water}} = 7:3\) (20L) for 30 days at room temperature. Then, it was filtered and the filtrate was concentrated under reduced pressure to obtain extract 800g, which was dispersed in 1000mL water and extracted with n-hexane, ethyl acetate and dichloromethane(3×1000mL) respectively. The organic layers were concentrated under reduced pressure to obtain three different polar extractions, which were dried in vacuum drying oven at 60°C for 1 hour to obtain the samples for biological screen.

**Preliminary in vitro anticancer evaluation**

The anticancer activity of obtained extractions(n-hexane extraction, ethyl acetate extraction and dichloromethane extraction) was evaluated against gastric cancer, breast cancer

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**Fig. 1.** New sterols isolated from the leaves of *Podocarpus nagi*
(MCF-7), lung cancer (A549) and Hela cell lines using the counting kit-8 (CCK-8) method. The evaluation processes were described elsewhere with some modifications. Briefly, the three extractions (n-hexane extraction, ethyl acetate extraction and dichloromethane extraction) were dissolved in DMSO at a starting concentration of 6.5 mg/mL, 16.9 mg/mL and 7.8 mg/mL, respectively for below using.

**The procedure for anticancer evaluation**

The target cancer cell lines were seeded in 96-well plates (5000 cells/well) with 100 μL DMEM supplemented with 10% fetal bovine serum, and cultured at 37 °C in a humidified CO₂ incubator (95% air, 5% CO₂) for 24 h. While the cell lines grew to 90% in logarithmic growth, the culture medium was removed from each well, and 100 μL fresh DEME was added to each well. Then, 10 μL above prepared solutions of different extractions were added into each well (every concentration was repeated for 5 times) and the plates were incubated for another 48 h at 37 °C. Subsequently, 10 μL CCK8 was added to each well, and the plates were cultured at 37 °C for another 4 h. The optical density was measured at a wave-length of 450 nm on an ELISA microplate reader. DMEM and DMSO solution (V/V: 10/1) was used as a negative control. The results were expressed as the inhibition calculated at the ratio \([1-(OD_{450 \text{ treated}}/OD_{450 \text{ negative control}})] \times 100\). By comparing the anticancer efficacy of three different extractions, ethyl acetate extract exhibited higher anticancer efficacy.

**Table 1. The inhibition rate of ethyl acetate extract to four tumor cell lines at 16.9 mg/mL**

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Inhibition (%)±SD (n=5)</th>
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<tbody>
<tr>
<td></td>
<td>Gastric cancer</td>
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<tr>
<td>16.9</td>
<td>97.04±0.52</td>
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</tbody>
</table>

**Fig. 2. The processes of extraction of the Podocarpus nagi kernel meal**

1. soaked in EtOH/H₂O(V/V: 7:3) for 1 month
2. filtered, and the filtrate was concentrated
   - dispersed in water
   - aqueous solution
     1. extracted using different solvents
     2. concentrated
       - in vitro anticancer screen
       - effective extraction

Podocarpus nagi kernel meal

**In vitro anticancer screen**

<table>
<thead>
<tr>
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<th>Three extractions</th>
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<tbody>
<tr>
<td></td>
<td>n-Hexane extraction</td>
<td>Ethyl acetate extraction</td>
<td>Dichloromethane extraction</td>
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</table>

**Effective extraction**
efficacy against gastric cancer, breast cancer (MCF-7), lung cancer (A549) and Hela cell lines. The results showed in table 1.

**CONCLUSION**

In this work, we obtained three different polar extractions from the *P. nagi* kernel meal and evaluated their anticancer efficacy against gastric cancer, breast cancer (MCF-7), lung cancer (A549) and Hela cell lines. The results showed that the ethyl acetate extraction exhibited higher anticancer activity against four listed cancer cell lines, which provided the scientific guidance for the subsequent work. Now we will focus on isolation and anticancer evaluation of this extraction for studying its material basis.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

3. Yang Y, Yong JP, Lu CZ. Chemical and biological progress of *Podocarpus nagi*. Biomedical Research and Reviews; **2**: 1-5 (2019).
8. Yong JP, Lu CZ, Zhang SB. Preparation method and use of *Podocarpus Nagi* kernel oil. AU innovation patent. 2020100726 (2020)