# Chemical Constituents of the Aerial Parts of Scoparia dulcis and Anti-cancer, Anti-inflammatory Activities

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# Chemical Constituents of the Aerial Parts of Scoparia dulcis and Anti-cancer, Anti-inflammatory Activities

**Nguyen Ngoc Thanh Tin[1], Nguyen Dang Thanh Truc[1], Hoang Thi Thu Hang[1,4], Pham Thi Nhat Trinh[2,4], Tri Duc Lam[3] and Le Tien Dung[5,6,*]**

[1]Nguyen Thong High School, Vinh Long Province, Vietnam

[2]Faculty of Natural Sciences, Tien Giang University, Tien Giang Province, Vietnam

[3]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

[4]Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

[5]Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam;

[6]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam;

*Email: inpcdung@yahoo.com, labasm2013@gmail.com

**Abstract.** In Vietnam, the medicinal plant *Scoparia dulcis* is used for the treatment of bronchitis, gastric disorders, antidiabetes, hypertension, hepatitis etc. In this paper, experiments was designed to evaluate in vitro anticancer and anti-inflammatory activities and isolate phytochemicals from methanol extracts of *Scoparia dulcis* whole plants. The chemical investigation of methanol fraction of Scoparia dulcis led to the isolation of benzoxazinone (1), phenylethanoid (2), flavone (3), and lignan (4) glycosides. The bioactivity results indicated that crude ethanol and ethyl acetate extracts had potent cytotoxic activity toward the HepG2 cancer cell with IC50 of 47.03 µg/mL and 36.04 µg/mL, respectively. Interestingly, crude ethanol and ethyl acetate extracts had the NO inhibitory activity, with inhibition of 56.9 % or 74.7 % at 30 µg/mL and 97.3% or 82.9 % at 100 µg/mL, respectively. In addition, the hexane extract at concentrations of 100 µg/ml demonstrated anti-inflammatory activity through the inhibition of nitric oxide production of 76.7%.

## 1. Introduction

Fruits, herbs and aromatic plants are important sources of phenolic compounds such as phenolic acids, flavonoids and anthocyanins, which have attracted considerable attention for their application in natural biomaterials [1-4]. *Scoparia dulcis*, (family: Scrophulariaceae), a perennial herb which is commonly found in tropical and subtropical regions around the world. It has been used to treat bronchitis, gastric disorders, antidiabetes, hypertension, hepatitis in Vietnamese folk medicine [5]. The results of previously phytochemical investigation indicated that this plant contained flavonoids,

diterpenoids, triterpenoids, sterol, benzoxazinoids etc [6-10]. Morever, *Scoparia dulcis* possessed antioxidant, anti inflamatory, analgesic, anti cancer, antidiabetic activities [11-15]. As parts of our continuing studies on phytoconstituents from Vietnam medicinal plants [16-19], we report the anti-cancer and anti-inflammatory activities and the separation and characterization of four glycoside compounds of methanol fraction.

## 2. Experimental

### 2.1. General experimental procedures

Specific rotation were measured on digital polarimeter (Kruss, Hamburg, German). NMR spectra were recorded on a Bruker AM500 FTNMR spectrometer (Bruker, Karlsruhe, Germany), Institute of Chemistry (VAST). ESI-MS were performed on a MicroOTOF-Q mass spectrometer (Bruker, Karlsruhe, Germany), University of Science (National University, HoChiMinh city, Vietnam). TLC was performed on silica gel 60 F254 (Merck, Darmstadt, Germany) and RP-18 F254S plates (Merck, Darmstadt, Germany). The zones were detected by $H_2SO_4$/EtOH. Column chromatography was performed on silica gel (Merck, Darmstadt, Germany), ODS (Merck, Darmstadt, Germany) and Sephadex LH- 20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

### 2.2. Plant materials

Whole plants of *Scoparia dulcis* were collected in Vinhlong province, Vietnam in June, 2018. A voucher specimen (015842) has been deposited in Institute of Applied Materials Science (VAST) by Dr. Luu Hong Truong.

### 2.3. Extraction and isolation

The aerial parts of *S.dulcis* (4.5 kg) was extracted at room temperature with ethanol 96% (5x10 L) for 4 weeks. The crude extract was concentrated to dryness in rotary evaporator at $60^0$ C to yield a residue (280 g). The residue was chromatographed on silica gel column to give n-hexane, ethyl acetate and methanol soluble fractions. The methanol fraction (126.7 g) was fractionated by column chromatography on silica gel (EtOAc: MeOH:$H_2O$=25:1:1 to 5:1:1) to yield three fractions (SDM1–3). Fraction SDM2 was chromatographed eluting with $CHCl_3$: MeOH (30:1 to 8:1) to obtain eight subfractions (SDM2.1-8). Subfraction SDM2.7 was chrommatographed on a silica gel column eluting with $CHCl_3$:MeOH (8:1 to 5:1) to obtain six subfractions (SDM2.7.1-6). Fraction SDM2.7.5 was rechromatographed on a silica gel column eluting by $CHCl_3$ : MeOH (8:1) and then followed by sephadex LH20 column (MeOH) to give **1** (218 mg). Fraction SDM2.7.6 was passed over Sephadex LH 20 column (MeOH) to give three fractions. Subfraction SDM2.7.6.2 was repeatedly purified through a ODS column (MeOH:$H_2O$ = 1:1) to give **2** (18.2 mg). Fraction SDM2.7.3 was subjected to a ODS comlumn (MeOH:$H_2O$=1:3) as eluent to give seven subfractions (SDM2.7.3.1-7). Subfraction SDM2.7.3.1 was purified through sephadex LH20 (MeOH), then subjected to a silica gel column eluting with $CHCl_3$ : MeOH (6:1) to obtain **3** (8.0 mg). Using a Sephadex LH-20 column (MeOH) to purify subfraction SDM2.7.3.4 and then followed by a ODS column eluting MeOH:$H_2O$ (1:2) to obtain **4** (30 mg).

### 2.4. Cytotoxicity assay

Human hepatocarcinoma HepG2 cells were maintained in Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum, 100 units/mL penicillin, and 10 μg/mL streptomycin at 37 ℃ and 5 % $CO_2$. Cell viability was assessed after the incubation with extracts at a concentration from 6 - 100 μg/mL for 24h. The plates were read immediately at 540 nm on a microplate reader. All the experiments were performed three times and the mean absorbance values were calculated.

### 2.5. Anti-inflammatory asay

RAW 264.7 macrophages were pretreated for 3 h with extracts, and treated for 24 h with LPS. The nitrite concentration in the medium was measured as an indicator of NO production by the Griess reaction. The inhibitory effect on the NO production was estimated by calculating the concentration ratio of nitrite produced in the cells treated with/without extracts.

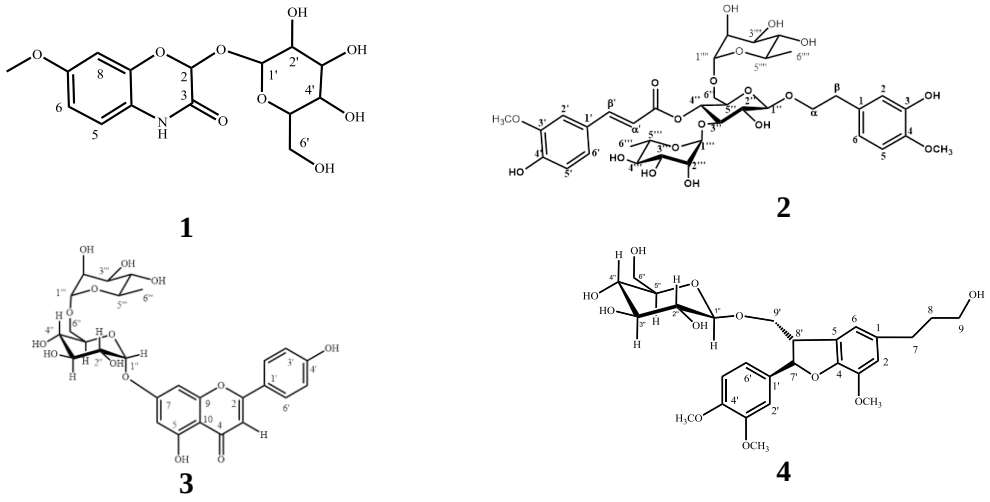

**Fig.1**. Isolated compounds (1-4)

## 3. Result and Discussion

The $^1$H NMR spectrum of **1** indicated the presence of a 1,2,4-trisubstituted benzene; one methoxyl group; one acetal proton; one anomeric proton as well as oxymethine proton of a glucose varied from 3.23 to 3.90 ppm. The correlation observed between H-2 $\delta_H$ 5.74 with C-3 $\delta_C$ 160.7 and C-8a $\delta_C$ 142.9 in HMBC of **1** indicated that **1** possess 1,4-benzoxazin-2-hydroxy-3-one skeleton with C-7 bearing methoxy.[16] The glucose moiety was located at C-2 of benzoxazinone framework due to the HMBC correlations of H-2 with C-1'. On the basis of the above evidence, compound **1** was indicated as 2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one 2-O-β-D-glucopyranoside [20,21].

The NMR spectrum of **2** exhibited the resonance of 37 carbon signals including two benzen rings, one double bond, an ethyloxyl moeity, one carbonyl carbon along with three sugar moieties consisting of one glucose and two rhamnose. The existence of feruloyl and 3-hydroxy-4-methoxy-phenylethanol moieties in **2** structure were proved due to correlations in HMBC spectrum. HMBC correlations between H-4'' with carbonyl carbon $\delta_C$ 167.9 indicated that feruloyl moiety attached to glucose at C-4''. This was confirmed through lowfield shift of H-4'' at $\delta_H$ 5.03 (t, 9.5Hz, H-4''). Similarly, the attachment of 3-hydroxy-4-methoxy-phenylethanol to C-1'' was established due to the cross-peak between $H_2$-α with C-1'' of glucose moiety. The interglycosidic linkage between glucose C-6'' and rhamnose C-1'''' was determined through correlation between H-1''' with C-6'' in the HMBC experiment. The anomeric proton $\delta_H$ 5.21 correlated with glucose C-3'' in HMBC spectrum indicated this rhamnose to be connected at C-3'' of glucose. Thus, compound **2** was established as Ferruginoside C [22]. Compounds **3** and **4** were determined as isorhoifolin and 3,4'-O-dimethylcedrusin 9'-O-glucopyranoside, respectively (Fig.1) [23,24].

The bioactivity results (Table 1 and 2) indicated that crude ethanol extract and ethyl acetate fraction had potent cytotoxic activity toward the HepG2 cancer cell with $IC_{50}$ of 47.03 µg/mL and 36.04 µg/mL, respectively. Interestingly, crude ethanol and ethyl acetate extracts had the NO inhibitory activity, with inhibition of 56.9 % or 74.7 % at 30 µg/mL and 97.3% or 82.9 %at 100 µg/mL, respectively. In addition, the hexane extract at concentrations of 100 µg/ml demonstrated anti-inflammatory activity through the inhibition of nitric oxide production of 76.7%.

**Table 1**. Cytotoxicity of extracts against HepG2 cell

| Samples | $IC_{50}$ (µg/mL) |
|---|---|
| Hexan fraction | ≥100 |
| Ethyl acetat fraction | 36.04 |
| Methanol fraction | ≥100 |
| Paclitaxel | 2.9 |

**Table 2**. Inhibition values of extracts on LPS-stimulated NO production in RAW 264.7 macrophages

| Samples | Concentration | Inhibition NO production (%) | Cell viability (%) |
|---|---|---|---|
| Crude extract | 30 µg/mL | 56.9±0.84 | 68.3±1.0 |
| | 100 µg/mL | 97.3±0.4 | 38.4±1.0 |
| Hexan fraction | 30 µg/mL | 27.4±0.7 | 103.8±2.1 |
| | 100 µg/mL | 76.7±0.4 | 95.8±0.9 |
| Ethyl acetat fraction | 30 µg/mL | 74.7±0.61 | 58.5±0.5 |
| | 100 µg/mL | 82.9±0.2 | 33.4±1.5 |
| Methanol fraction | 30 µg/mL | 14.8±0.5 | 109.4±1.3 |
| | 100 µg/mL | 47.3±0.8 | 81.2±1.6 |
| Cardamonin | 0.3 µM | 17.1±0.8 | 85.8±0.1 |
| | 3.0 µM | 96.6±0.6 | 85.8±0.1 |

## 4. Conclusion

From methanol fraction of *Scoparia dulcis*, 2-hydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one 2-O-β-D-glucopyranoside (**1**), ferruginoside C (**2**), isorhoifolin (**3**) and 3,4'-O-dimethylcedrusin 9'-O-glucopyranoside (**4**) were isolated. Crude ethanol and ethyl acetate fractions showed cytotoxic activity toward the HepG2 cancer cell with $IC_{50}$ of 47.03 µg/mL and 36.04 µg/mL, respectively. In addition, crude ethanol and ethyl acetate extracts at 30 and 100 µg/mL inhibited NO production. The hexane extract at concentration of 100 µg/ml showed the inhibition of nitric oxide production of 76.7%.

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