Rhoifolin; A Potent Selective Flavone for the *In-Vitro* Treatment of Cancer

**ABSTRACT**

**Aims:** To investigate the cytotoxic activity of rhoifolin against different cancer cell lines

**Study Design:** isolation, identification and cytotoxic activity evaluation.

**Place and Duration of Study:** Faculty of Pharmacy, Ain Shams University and Al-Azhar University, between October, 2010 and January, 2011.

**Methodology:** Rhoifolin, Apigenin 7-O-β neohesperidoside was isolated in a copious amount from the leaves of *Chorisia crisplflora* (Bombaceae). Its identity was unambiguously confirmed via different spectroscopic methods and Viability assay test was used to evaluate its cytotoxic activity.

**Results:** It exhibited potent anticancer activities, nearly similar to that of vinblastine, when evaluated against human epidermoid larynx (Hep 2) and human cervical (HeLa) carcinoma cell lines. Promising activities were also obtained against hepatocellular (Hep G2), colon (HCT) and fetal human lung fibroblast (MRC-5) carcinoma cell lines. A unique effect of rhoifolin was in having no cytotoxic activity against healthy normal cells which indicates a high selectivity.

**Conclusion:** The findings of this study showed that rhoifolin could be futurally used as an ideal anticancer agent where it is toxic to malignant cells with no toxicity in normal cells.

**Keywords:** Rhoifolin; *Chorisia crisplflora*; Bombaceae; spectroscopic methods; cytotoxic activity

1. **INTRODUCTION**

The introduction of active agents derived from nature into the cancer armamentarium has changed the natural history of many types of human cancer. Throughout medical history novel plant-derived compounds were of great significance to cancer therapy. Examples are vinblastine and vincristine from
Catharanthus roseus (Apocynaceae). (Kalidass et al, 2010). Currently, one of the major cancer treatments is chemotherapy. Most of chemotherapy drugs such as vincristine, paclitaxel, and etoposide (ET) cannot discriminate between cancer cells and noncancer cells, many normal cells are also killed during the process of chemotherapy. This nonspecific cytotoxicity damages the patient’s immune system and generates many side effects such as neutropenia, vomiting, hair loss, peripheral neurotoxicity, etc. (Perry et al, 1976 and Einzig et al, 1991). Apigenin nucleus is a cancer chemopreventive agent. It inhibits cell proliferation in cancer cell types (Kanno et al, 2004). A number of the biological effects of apigenin in numerous mammalian systems in-vitro as well as in-vivo are related to its antioxidant effects and its role in scavenging free radicals. Furthermore, it exhibits anti-mutagenic, anti-inflammatory, antiviral, and purgative effects (Kanno et al, 2004). The first report about apigenin in human cervical carcinoma HeLa cells demonstrated apigenin inhibited the growth through an apoptotic pathway (Zheng et al, 2005). The effects of apigenin on lung cancer cells were evaluated, apigenin inhibited A549 lung cancer cell proliferation and vascular endothelial growth factor (VEGF) transcriptional activation in a dose-dependent manner (Li ZD, et al, 2007).

So far, nothing has been documented about the cytotoxic effect of apigenin 7-neohesperidoside (rhoifolin). Wherefore the anticancer activity of this compound was investigated to develop a first building block for the construction of a new anticancer drug.

We reported here for the first time the high potent and selective anticancer activity of rhoifolin against different types of cancerous cell lines beside the non toxic effect towards normal cells.

2. MATERIALS AND METHODS

2.1. Plant Material

Chorisia leaves were collected from Zoo Garden in Giza, Egypt, 2010 and were authenticated by Prof. Dr Abdel Salam El Noyehy, Prof. of Taxonomy, Faculty of Science, Ain Shams University, Cairo, Egypt. Voucher specimen was deposited at the herbarium of Pharmacognosy Department (voucher specimen number: CCB-73), Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The leaves were dried in shade and reduced to a fine powder.
2.2. Extraction And Isolation

Powder of air dried leaves of *Chorisia crispiflora* (1 kg) was extracted with 70 % ethanol on cold. The extract was completely dried and dissolved in a small amount of water and partitioned with *n*-hexane, ethyl acetate, butanol successively. The left water residue was completely dried and extracted with methanol on hot (40°C). The methanolic extract upon concentration yielded yellow crystals of rhoifolin (8.3 g). Purification to the crystals was achieved by crystallization.

2.3. Instruments And Materials For Phytochemical Investigation

Chromatographically pure materials 1 mg each were dissolved in analytically pure methanol then subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. *AlCl₃, AlCl₃/HCl*, fused *NaOAc / H₃BO₃* and *NaOMe* reagents were separately added to the methanolic solution of investigated material and UV measurements were then carried out.

The NMR spectra were recorded on a Varian Mercury VX-500 NMR spectrometer. 1H- spectra run at 300 MHz and 13C- spectra were run at 75.46 MHz in deuterated dimethylsulphoxide (DMSO- d6).

Roifolin: Apigenin 7-O-β neohesperidoside, *C₂₇H₃₀O₁₄*, yellow needles, m.p. 250-265°C.

IR \( \nu_{\text{max}} \) (KBr): 3388 (OH), 1657 (\( \alpha, \beta \)-unsat. CO), 1605, 1497, and 1488 (arom. C=C), 1249, 1178, 1074 (glycosidic C–O) cm\(^{-1}\).

UV \( \lambda_{\text{max}} \) (log \( \varepsilon \)) (MeOH): 266 (4.20), 336 (4.30) nm; MeONa): 267 (4.20), 387 (4.40) nm; (NaOAc): 257 (4.20), 266 (4.20), 391 (4.40) nm, (NaOAc + H₃BO₃): 268 (4.20), 339 (4.30) nm; (AlCl₃): 275 (4.20), 299 (4.10), 350 (4.20), 385 (4.20) nm, (AlCl₃ + HCl): 276 (4.20), 298 (4.10), 345 (4.20), 382 (4.10) nm.

\(^{1}\)H-NMR, DMSO- d₆ \( \delta \) ppm: 7.91(2H, d, \( J=8.8 \) Hz, H-2’,6’), 6.92(2H, d, \( J=8.8 \) Hz, H-3’,5’), 6.84 (1H, d, \( J=2.0 \) Hz, H-8), 6.80 (1H, s, H-3), 6.33(1H, d, \( J=2.0 \) Hz , H-6), 5.20(1H, singlet like, H-1”), 5.08 (1H, d, \( J=7.3 \) Hz, H-1”), 1.16(3H, d, \( J=6.3 \)Hz, H3-6”’).
\[ ^{13}\text{C-NMR, DMSO-d}_6 \delta \text{ pp: 182.1-C4, 164.4-C2, 162.6-C7, 161.7-C4', 161.1-C5, 157.1-C9, 128.7-C2',6', 120.9-C1', 116.2-C3',5', 105.5-C10, 103.2-C3, 99.4-C6, 94.6-C8, Sugar proton: 101.01-C1``', 99.8-C6, 98.2-C1``', 95.01-C8, 77.6-C2``', 77.4-C3``, 76.8-C5``, 72.3-C4``', 71.0-C2``', 70.8-C3``', 70.1-C4``', 68.8-C5``, 60.9-C-6``, 18.5-C-CH3} \]

2.4. Mammalian Cell Lines

Vero cells (Normal kidney cells)

Carcinoma cell lines: Hep2 (human epidermoid larynx carcinoma cells), HeLa cells (human cervical carcinoma cells), Hep G2 (human hepatocellular carcinoma), HCT (human colon carcinoma cells) and MRC-5 (fetal human lung fibroblast cells).

All cell lines of a well differentiated carcinoma were obtained from the American Type Culture Collection (ATCC)

2.5. Chemical Used

Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye (Sigma, St.Louis, Mo., USA)

DMEM, RPMI-1640, FBS, HEPES buffer solution, L-glutamine, gentamycin and 0.25 % Trypsin-EDTA (Bio Whittaker ®Lonza, Belgium). Crystal violet stain (1%)

2.6. Cytotoxicity Evaluation Using Viability Assay

Cell toxicity was monitored by determining the effect of the test sample on cell morphology and cell viability through viability assay (Vijaya et al, 2004 and Mosmann, 1983).

3. Results And Discussion

3.1. Identification of the Compound

Pure material of rhoifolin was obtained as an amorphous light yellow powder, which appeared as a dark purple spot on Paper chromatography (PC) and turned yellow upon exposure to ammonia vapors, under short UV light (254 nm).

Confirmation of the compound was achieved through UV shift reagents, 1HNMR, 13CNMR and HMBC correlation (Fig. 1).
3.2. Cytotoxic Activity Of The Compound

The tested compound showed marked toxic effects to the cancerous cell lines. It exerted cytotoxic activity to Hep 2 and HeLa cell lines at IC50 5.90, 6.2 µg respectively (Fig. 2 B & C). HepG2 is affected but to a lesser extent by the compound at IC50 22.6 µg (Fig. 2-D). The least potent activities were to HCT and MRC-5 at IC50 34.8 and 44.6 µg respectively (Fig. 2 E &F).

Historically natural products have been an important source of anticancer drugs. 60% of currently used anticancer agents are of natural origin, derived from plants, marine organisms and is a useful tool for the discovery of new potential anticancer agents from natural products. One of the important criteria for a therapeutic drug for cancer is to have minimum or no side effects on normal body cells of patients undergoing chemotherapy. This invariably implies that the drug should not only have high potent activity at lower concentrations but also should exhibit high degree of selectivity.

Thus, development of novel selective drugs is an important and challenging task, and understanding the biological differences between normal and cancer cells is essential for achieving this goal.

The present in-vitro study showed the ability of rhoifolin to exhibit a high degree cytotoxic activity to cancerous cells with great selectivity, where, as it is clear, that the compound has no cytotoxic activity against mammalian normal cells Table 1, Fig 2A.

Table 1 showed that the compound of interest exhibited high cytotoxic activity to laryngeal cancer cells, at a very low IC50= 5.9 µg followed by cervical at IC50= 6.2 µg, both are nearly similar to that of vinblastine. Hepatic cell line was also affected but at a lesser extent by the toxicity of rhoifolin at IC50 22.6 µg. The colon at and the fetal human lung fibroblast cell lines are affected at IC50 34.8 and 44.6 µg respectively.

As the Selective index (SI) demonstrates the differential activity of a pure compound, the greater the SI value is, the more selective it is. An SI value less than 2 indicates general toxicity of the pure compound (Koch et al, 2005).
Based on this, the SI data shown in Table 2 indicates that rhoifolin exhibits a very high degree of cytotoxic selectivity at SI greater than 8.47 for laryngeal cell lines, followed by 8.06 in cervical 2.21 in hepatic carcinoma cell lines. The other two carcinoma cell lines; colon and fetal human lung fibroblast are of little SI.

4. CONCLUSION

Interestingly, this present study, showed the following advantages of rhoifolin:
1. Potent cytotoxic effect nearly similar to that of vineblastine which may become a good therapeutic strategy to its use as an antagonist for treatment of this dreaded disease, especially laryngeal, cervical and hepatic cancer.
2. It is considered as an ideal anticancer agent where it is toxic to malignant cells with no toxicity in normal cells. However, currently there are limited numbers of such agents available for clinical use.
The mechanisms behind its respective anticancer effect are now under investigation to pave a way for a discovery of a new cancer therapeutic agent.

ACKNOWLEDGEMENT

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COMETING INTEREST

The author declares that there is no conflict of interest t relevant to this study.

REFERENCES


**Figures**

**Figure 1.** Rhoifolin; HMBC correlations of Apigenin 7-O-β neohesperidoside

**Figure 2.** Cytotoxic activity of rhoifolin on different human cell lines:
(A) Mammalian vero cell line (normal cell), (B) Hep2 (human epidermoid larynx carcinoma cells), (C) HeLa cells (human cervical carcinoma cells), (D) Hep G2 (human hepatocellular carcinoma), (E) HCT (human colon carcinoma cells) and (F) MRC-5 (fetal human lung fibroblast cells)
Table 1. IC$_{50}$ in microgram and micromole of rhoifolin and vinblastine on the carcinoma cell lines.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Rhoifolin</th>
<th>Vinblastine</th>
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<tbody>
<tr>
<td></td>
<td>µg</td>
<td>µM</td>
</tr>
<tr>
<td>MRC-5</td>
<td>44.6</td>
<td>0.0770</td>
</tr>
<tr>
<td>HCT</td>
<td>34.8</td>
<td>0.0601</td>
</tr>
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<td>HepG2</td>
<td>22.6</td>
<td>0.0390</td>
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<td>HeLa</td>
<td>6.20</td>
<td>0.0107</td>
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<tr>
<td>Hep2</td>
<td>5.90</td>
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Table 2. Selective indices of rhoifolin on carcinoma cell lines.

<table>
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<th>Cell lines</th>
<th>SI</th>
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<tbody>
<tr>
<td>MRC-5</td>
<td>&gt; 1.12</td>
</tr>
<tr>
<td>HCT</td>
<td>&gt; 1.43</td>
</tr>
<tr>
<td>HepG2</td>
<td>&gt; 2.21</td>
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<tr>
<td>HeLa</td>
<td>&gt; 8.06</td>
</tr>
<tr>
<td>Hep2</td>
<td>&gt; 8.47</td>
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