Rhoifolin; A Potent Antiproliferative Effect On Cancer Cell Lines

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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 crispiflora* (Bombaceae). Its identity was unambiguously confirmed via different spectroscopic methods (UV, ^{1}HNMR, ^{13}CNMR and HMBC) 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 larynex (Hep 2) and human cervical (HeLa) carcinoma cell lines. Promising activities were also obtained against hepatocellular (Hep G2), colon (HCT-116) 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 (Vero cells) which indicates a high selectivity of the compound selected.
Conclusion: The findings of this study showed that rhoifolin could be used as an ideal anticancer agent. It discriminates between cancerous and non cancerous cell as it kills only the former one. So the side effects which may appear during chemotherapy could be overcome.

Keywords: Roifolin; Chorisia crispiflora; 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. As examples of these compounds are vinblastine and vincristine; *Catharanthus roseus* family Apocynaceae (Kalidass *et al.,* 2010). Currently, one of the major cancer treatments is chemotherapy. Most of the chemotherapeutic drugs such as vincristine, paclitaxel, and etoposide (ET) cannot discriminate between cancer and non-cancer 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).

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Rhoifolin is apigenin 7-O-β neohesperidoside. It was reported that rhoifolin has lots of pharmacological actions. It exerts its anti-diabetic effect through enhanced adiponectin secretion, phosphorylation of insulin receptor-*β*, and GLUT4 translocation (Rao *et al*., 2011). It has an antiinflammatory action via multi-level regulation of inflammatory mediators (Eldahshan and Azab, 2012). Rhoifolin produced no change in hypoxic pulmonary vasoconstriction, but decreased cardiac output and aortic pressure (Occhiuto and Limardi, 1994).

Apigenin nucleus is a cancer chemopreventive agent. It inhibits cell proliferation in cancer cell types (Sarkar and Li, 2004). Because of its potential antioxidant, anti-inflammatory, and anti-tumor properties, apigenin is considered as a candidate cancer chemopreventive agent (Birt *et al*., 1996; Birt *et al*., 1997; Lepley *et al*., 1996; Ross and Kasum, 2002).

Apigenin inhibited the growth through an apoptotic pathway in human cervical carcinoma HeLa cells (Zheng *et al*., 2005). Furthermore, apigenin inhibited A549 lung cancer cell proliferation and vascular endothelial growth factor (VEGF) transcriptional activation in a dose-dependent manner (Ling *et al*., 2005). However, Kawaii (Kawaii *et al*., 1999) reported the weak effect of rhoifolin against melanin pigment producing mouse melanoma, human T-cell leukemia, human lung and lymph node metastatic carcinoma cell lines.

So far, nothing has been documented about the cytotoxic effect of rhoifolin against human epidermoid larynex (Hep 2), human cervical (HeLa), hepatocellular (Hep G2), colon (HCT-116) and fetal human lung fibroblast (MRC-5) carcinoma cell lines, so the antineoplastic activity of this compound was investigated against these types of cancer cell lines. We reported here for the first time the high potent and selective antitumor activity of rhoifolin against several types of cancer cell lines to develop a preliminary building block for the construction of a new anticancer drug.

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 in 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 milled to a fine powder.

2.2. Extraction and Isolation

Powder of air dried leaves of *Chorisia crispiflora* (1 kg) was extracted with 70 % ethanol at room temperature. The extract was entirely dried and dissolved in a small amount of water and partioned with *n*-hexane, ethyl acetate and butanol successively. The aqueous water residue was totally dried and extracted with methanol at 40ºC. 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 next subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. $AlCl_3$, $AlCl_3$/HCl, fused NaOAc / H_3BO_3 and NaOMe reagents were separately added to the methanolic solution of investigated material and UV measurements were later carried out.

The NMR spectra were recorded on a Varian Mercury VX-500 NMR spectrometer. ^{1}H- spectra ran at 300 MHz and ^{13}C- spectra were run at 75.46 MHz in deutrated dimethylsulphoxide (DMSO-d_6).

Rhoifolin: Apigenin 7-O-β neohesperidoside, $C_{27}H_{30}O_{14}$, yellow needles, m.p. 250-265ºC.

IR vmax (KBr): 3388 (OH), 1657 (α, β-unsat. CO), 1605, 1497, and 1488 (arom. C=C), 1249, 1178, 1074 (glycosidic C–O) cm^{-1}.

UV λmax (log ε) (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 + H_3BO_3): 268 (4.20), 340 (4.30) nm; (AlCl3): 275 (4.20), 299 (4.10), 350 (4.20), 385 (4.20) nm, ($AlCl_3$ + HCl): 276 (4.20), 298 (4.10), 342 (4.20), 382 (4.10) nm.

^{1}H-NMR, DMSO-d_6 δ 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), 5.08(1H, singlet like, H-1```), 5.20 (1H, d, *J*= 7.3 Hz, H-1``), 1.16(3H, d, *J*=6.3Hz, H3-6```).

^{13}C-NMR, DMSO-d_6 δ ppm: 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`, 116.2C-3`,5`, 105.5-C10, 103.2-C3, 99.4-C6, 94.6-C8 , Sugar proton: 100.5-C1``, 98.2-C1```, 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-CH_3.

2.4. Mammalian Cell Lines

Vero cells (Normal kidney cells).

Carcinoma cell lines: Hep2 (human epidermoid larynex carcinoma cells), HeLa cells (human cervical carcinoma cells), Hep G2 (human hepatocellular carcinoma), HCT-116 (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 viability through the viability test (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, ^{1}HNMR, ^{13}CNMR 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 IC_{50} 5.90 and 6.2 µg/mL respectively (Fig. 2 B & C). HepG2 is affected but to a lesser extent by the compound at IC_{50} 22.6 µg/mL (Fig. 2-D). The least potent activities were to HCT-116 and MRC-5 at IC_{50} 34.8 and 44.6 µg/mL respectively (Fig. 2 E & F).

Historically natural products have been an important source of antineoplastic drugs. Sixty percent of currently used antitumor 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 a 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 IC_{50}= 5.9 µg/mL followed by cervical at IC_{50}= 6.2 µg/mL, 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 IC_{50} 22.6 µg/mL. The colon and the fetal human lung fibroblast cell lines are affected at IC_{50} 34.8 and 44.6 µg/mL respectively.

The selectivity index (SI) was defined as the ratio of the IC_{50} obtained from the experiment on normal cells vs. cancer cells. High selectivity was achieved when the SI was ≥ 3 (Prayong *et al.*, 2008). 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 and 2.21 in hepatic carcinoma cell lines. The other two carcinoma cell lines; colon and fetal human lung fibroblast are of little SI.

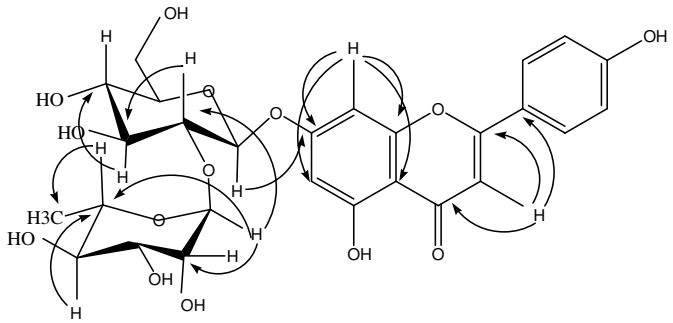

Fig. 1. HMBC correlations of Apigenin 7-*O*-β neohesperidoside (Rhoifolin)

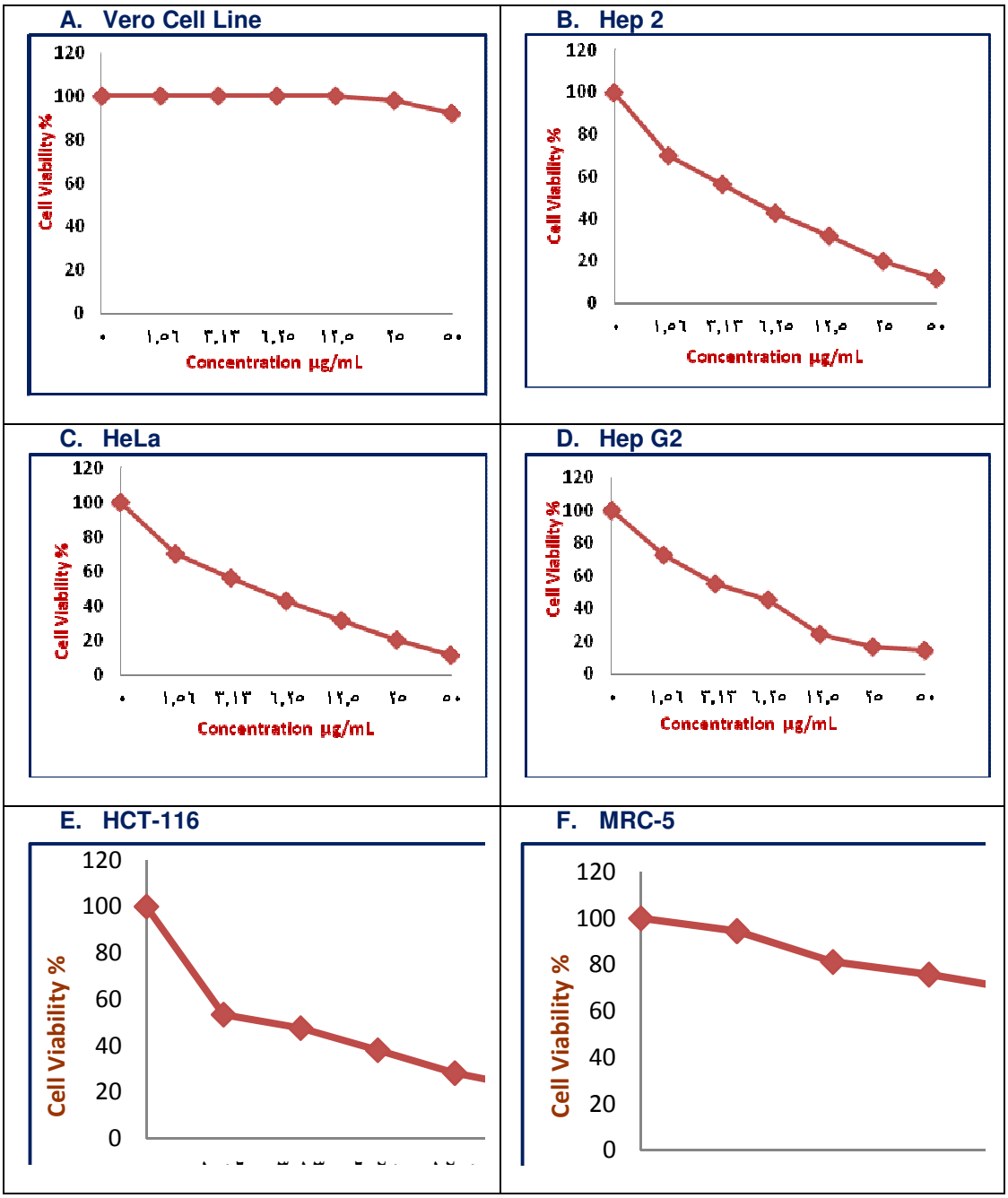

Fig. 2. Cytotoxic activity of rhoifolin on different human cell lines (the concentration in µg/mL): (A) Mammalian vero cell line (normal cell), (B) Hep2 (human epidermoid larynex carcinoma cells), (C) HeLa cells (human cervical carcinoma cells), (D) Hep G2 (human hepatocellular carcinoma), (E) HCT-116 (human colon carcinoma cells) and (F) MRC-5 (fetal human lung fibroblast cells).

Table 1. IC_{50} of rhoifolin and vinblastine on the carcinoma cell lines.

Cell lines	Rhoifolin Concentration		Vinblastine	
	µg/mL	µM	µg/mL	µM
MRC-5	44.6	0.0770	4.6	0.0055
HCT	34.8	0.0601	2.6	0.0031
HepG2	22.6	0.0390	4.6	0.0055
HeLa	6.20	0.0107	5.2	0.0063
Hep2	5.90	0.0101	4.6	0.0055

Table 2. Selective indices of rhoifolin on carcinoma cell lines.

Cell lines	SI
MRC-5	> 1.12
HCT	> 1.43
HepG2	> 2.21
HeLa	> 8.06
Hep2	> 8.47

4. CONCLUSION

Interestingly, this present study, showed the following advantages of rhoifolin:

1. Potent cytotoxic effect nearly similar to that of vinblastine 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 antitumor agent to specific cancerous cells where it is toxic to malignant with no toxicity to normal cells so it will be a good building unit for a new antitumor drug without side effects.

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 to a discovery of a new cancer therapeutic agent.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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