academicJournals

Vol. 7(20), pp. 1401-1405, 25 May, 2013
DOI: 10.5897/JMPR11.801
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Eupalitin from *Asparagus falcatus* (Linn.) has anti-cancer activity and induces activation of caspases 3/7 in human colorectal tumor cells

Raza Murad Ghalib[1]*, Sayed Hasan Mehdi[2], Rokiah Hashim[2], Othman Sulaiman[2], Fiona How Ni Foong[1], B. M. Khadeer Ahamed[3], Amin Malik Shah Abdul Majid[3] and Faiyaz Ahmed[4]

[1]Department of Chemistry, Kulliyaah of Science, IIUM, Kuantan, Malaysia.
[2]School of Industrial Technology, Universiti Sains Malaysia, Minden–11800, Pulau Pinang, Malaysia.
[3]Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden–11800, Pulau Pinang, Malaysia.
[4]Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries, Govt of India, Pudukkottai Road, Thanjavur 613 005, Tamil Nadu, India.

Accepted 13 May, 2013

3,5,4′-Trihydroxy-6,7-dimethoxy-flavone (Eupalitin) has been isolated from the leaves of *Asparagus falcatus* (Linn.). Anti-proliferation and apoptosis studies were conducted on eupalitin. Results showed that eupalitin exhibited significant cytotoxicity against human colorectal tumor cells. It is found that eupalitin induces the activation of caspases 3/7, a hallmark of apoptosis. The study suggests that the anti-proliferative property of eupalitin towards the human colorectal tumor cells may be probably due to its capability to induce apoptosis in cells.

Key words: 3,5,4′-Trihydroxy-6,7-dimethoxy-flavone, eupalitin, *Asparagus falcatus* (Linn.), cytotoxicity, colorectal cancer, caspases.

INTRODUCTION

It has been investigated that flavonoids may be effective in combating certain types of cancer (Sarin et al., 1976; Kupchan and Bauerschmidt, 1971; Zhao et al., 2010; Wen et al., 2010; Shao et al., 2010). The effect of flavonoids on cancer cells in various pharmacokinetics of a flavonolignan couple has also been studied (Zhao et al., 2010). Many flavones, isoflavones, and flavanones have been found to have antineoplastic activity and cytotoxicity (Edwards et al., 1979). A recent survey of literature showed that the flavonoids field is still very important to chemists, and their interest is increasing in screening the new flavonoids and their physiological activities. Several flavonoids have been shown to have potential as hepatoprotective agents such as dihydro kaempferol-3-rhamnoside, and 5, 7,3',5'-tetrahydroxy flavononol-3-rhamnoside showed some hepatoprotective natural products and their analogues as activity (Martini et al., 2004). The research for bioactive chemotherapeutic agents continues in preclinical development (Lee, 2004). Various phytochemicals originating from medicinal plants have been shown to have anti-tumor activities, and some of them are currently used in clinical trials (DaRocha et al., 2001). There is an increasing awareness that chemotherapeutic agents act primarily by inducing cancer cell death through the mechanisms of apoptosis (Lowe and Lin, 2000). Colorectal cancer is one of a major public health problems worldwide and its incidence is increasing annually (Dulabh et al., 2006; Hoff et al., 2001). Most physiologically related cell deaths in the body occur by an active process of self-destruction known as programmed

*Corresponding author. E-mail: raza2005communications@gmail.com. Tel. 0164526657.

cell death or apoptosis. The frequency of apoptosis could contribute to cell loss in tumors and promote tumors regression (Bold et al., 1997). One of the characteristic features of cancer cells is to escape from apoptosis pathways, so the induction of apoptosis is a brilliant approach to eradicate the cancerous cells without damaging the surrounding tissue (Naik et al., 1996). The treatment of cancer may benefit from the introduction of novel therapies derived from natural products.

Natural products have served to provide a basis for many of the pharmaceutical agents in current use in cancer therapy (Pietras and Weinberg, 2005). Eupalitin (Ghalib et al., 2010) (Figure 1) is a rarely found flavnoidic compound. Previous publications have reported it from *Mikania micrantha* (Wei et al., 2004), *Rudbeckia bicolor* (Kalidhar, 1990), *Flaveria chloraefolia* (Barron and Ibrahim, 1987), *Boerhaavia diffusa* Linn. (Pandey et al., 2005), *Artemisia lindleyana* (McCormick and Bohm, 1986) and *Rudbeckia serotina* (Waddell et al., 1985). An attempt is made to evaluate the *in vitro* anti-cancer property of eupalitin isolated from *Asparagus falcatus* (Linn.) against human colorectal tumor cells HCT116.

MATERIALS AND METHODS

Plant

The leaves of *A. falcatus* (Linn.), family Asparagaceae, were collected in December, 2009 from South Africa. Botanical identification of the plant material was performed by Taxonomist Mrs. Siti Nurdijati Baharuddin, School of Biological Sciences, USM, Malaysia. A voucher specimen has been deposited in the herbarium of the School of Biological Sciences, USM, Malaysia (No. 11129).

Extraction and isolation

The air dried and crushed sample (1.0 kg) was extracted with chloroform (5 × 3 L) under reflux for 3 × 3 h. The extract was concentrated to dryness on rotary evaporator under reduced pressure to give dry chloroform extract (120 g). The chloroform (60 g) was then chromatographed over silica gel (60 to 120 mesh, merck) column (8 × 140 cm) loaded in light petroleum ether. The column was eluted successively with light petroleum ether (4 L), light petroleum ether-diethylether (9:1 to 1:1, each 4 L), ethyl acetate (4 L), and ethyl acetate-methanol (9:1 to 1:1, each 4 L) as eluting solvents. The elutants obtained in solvent system ethyl acetate, ethyl acetate-methanol (9:1 to 4:1) on crystallization with chloroform-methanol (1:1) gave the crystals of the eupalitin (40 mg) (Ghalib et al., 2010). Furthermore, we got a very good amount (640 mg) of a solid compound from fractions obtained in light petroleum ether-diethylether (1:1) which was crystallized with chloroform-methanol and confirmed as Eupalitin by X-ray analysis (Figure 1) and by comparing with previously published data (Ghalib et al., 2010). Total yield of 680 mg eupalitin was obtained from 60 g extract.

Chemicals and reagents

Roswell Park Memorial Institute medium (RPMI) 1640 was purchased from ScienCell, USA. Trypsin and heat inactivated foetal bovine serum (HIFBS) were obtained from GIBCO, UK. Phosphate buffered saline (PBS), penicillin/streptomycin (PS) solution, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) reagent and 5-flurouracil were purchased from Sigma-Aldrich, Germany. All other chemicals used in this study were of analytical grade or better.

Cell lines and culture conditions

Human colorectal tumor (HCT116) cell line was purchased from American type culture collection (Rockvill, MD, USA). HCT116 cells were maintained in RPMI 1640 containing 10% HIFBS and 1% PS. Cells were cultured in 5% CO_2-humidifed atmosphere at 37°C.

Cells proliferation assay

Cytotoxicity of eupalitin was evaluated using MTT assay against human colorectal tumor cell line (HCT 116). The assay was carried out using the method described by Mosmann (1983) but with minor modification, following 48 h of incubation. Assay plates were read using the micro-titer plate reader (Hitachi U-2000, Japan) at 570 nm absorbance. 0.1% dimethyl sulphuroxide (DMSO) was used as negative control. 5-Flurouracil was used as positive control.

Apoptosis assay with caspases 3/7 activities

Following treatment with eupalitin, HCT 116 cells were subjected to caspase 3/7, activities measurement was done with Caspase-Glo assay kit (Promega, USA). In brief, after 3 h treatment, 100 µl of Caspase-Glo reagent was added to each well. The plate was then incubated for 30 min at room temperature. The luminescence which is proportional to net caspases activities was measured in a plate-reading luminometer (HIDEX, Finland). The experiments were performed in triplicate.

RESULTS AND DISCUSSION

In the present study, we report for the first time that eupalitin has significant ability to inhibit the proliferation of human colorectal cancer cells by activating the caspase 3/7 cascade. The anti-proliferative property was assessed using MTT assay, and the activity of caspases (cysteine-dependent aspartate-specific proteinase) which is known as an important biochemical feature in apoptotic signaling was further determined to investigate whether the apoptosis was induced by the eupalitin. Since caspases 3 and 7 are the main downstream effecter caspases that are present in most cell types and play an important role in the execution of apoptosis cell death by cleaving the cellular substrates (Kumar, 2007), we then compared its activity in treated and untreated control cells. The result showed that caspases 3 and 7 were significantly activated in the treated cells.

Cytotoxic potency of eupalitin was evaluated using MTT assay on HCT 116, MCF7 cell line. Eupalitin exhibited significant cytotoxicity against colorectal cancer (HCT 116) cell line with IC_{50} (concentration of test substance to achieve 50% inhibition) 22.9 µg/ml ($P < 0.01$). Figure 2 depicts the dose dependent inhibitory response

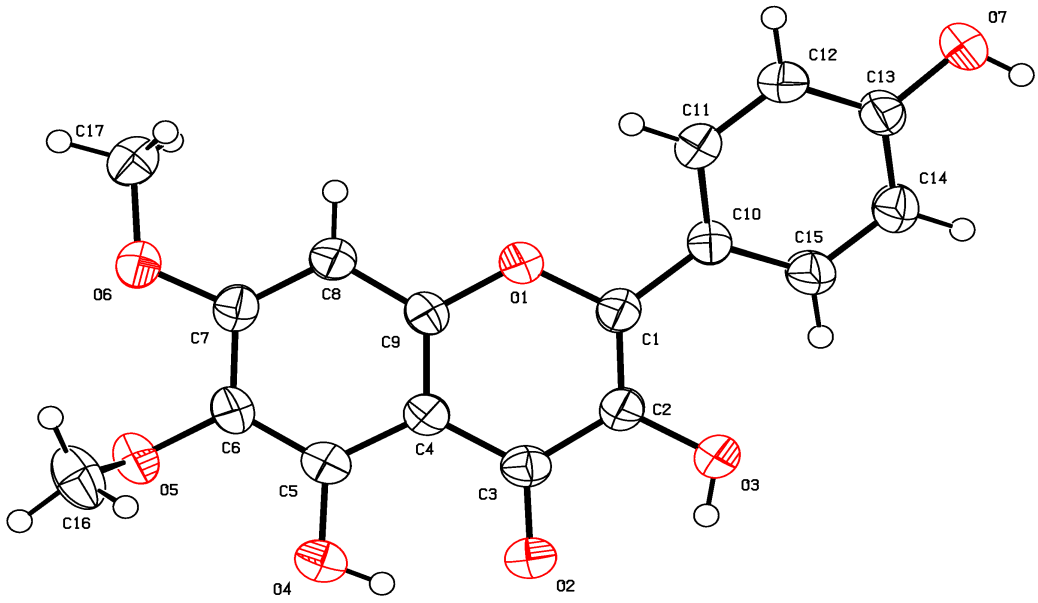

Figure 1. Ortep view of Eupalitin.

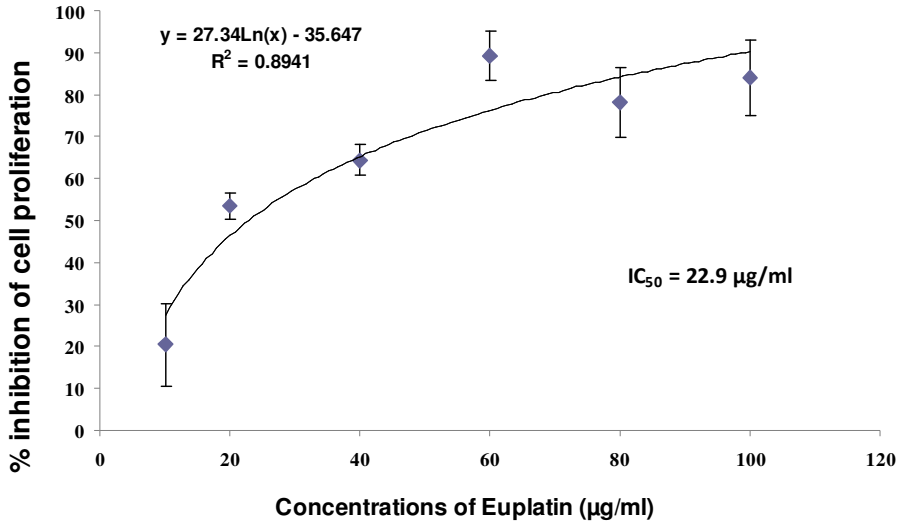

Figure 2. Inhibition of cell proliferation by eupalitin.

of cell proliferation due to the effect of eupalitin. On the other hand, the cytotoxic effect of 5-flurouracil on HCT 116 cells was very strong with IC_{50} 4.6 µg/ml (Figure 3). An attempt was made to identify whether the eupalitin induced inhibition of cell proliferation was due to induction of apoptosis.

After three hour treatment, eupalitin induced the appearance of caspases activities in HCT 116 cells in presence of Caspase-Glo reagent. From the results, it is conspicuous that eupalitin activates the promoter caspases that is, caspases 3/7. Figure 4 illustrates the induction of caspases activity by eupalitin. Our results demonstrated that eupalitin induced apoptosis as detected by the activation of caspases 3 and 7 in HCT 116 cells which seems to account for anti-proliferative activity determined by the MTT assay. The cytotoxic response of the HCT 116 cells to eupalitin was dose dependent.

ACKNOWLEDGEMENTS

We would like to acknowledge Universiti Sains Malaysia (USM) for the University Grant 1001/PTEKIND/8140152.

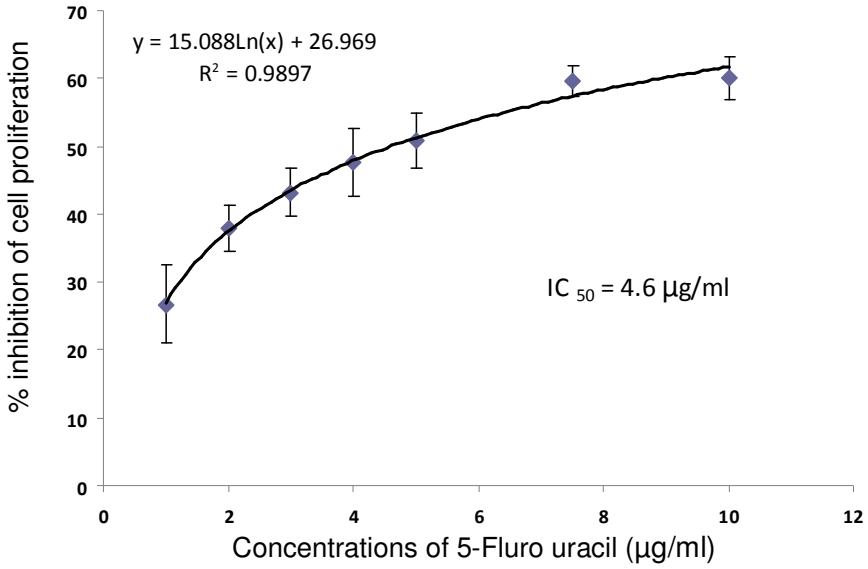

Figure 3. Inhibition of cell proliferation by 5-flurouracil.

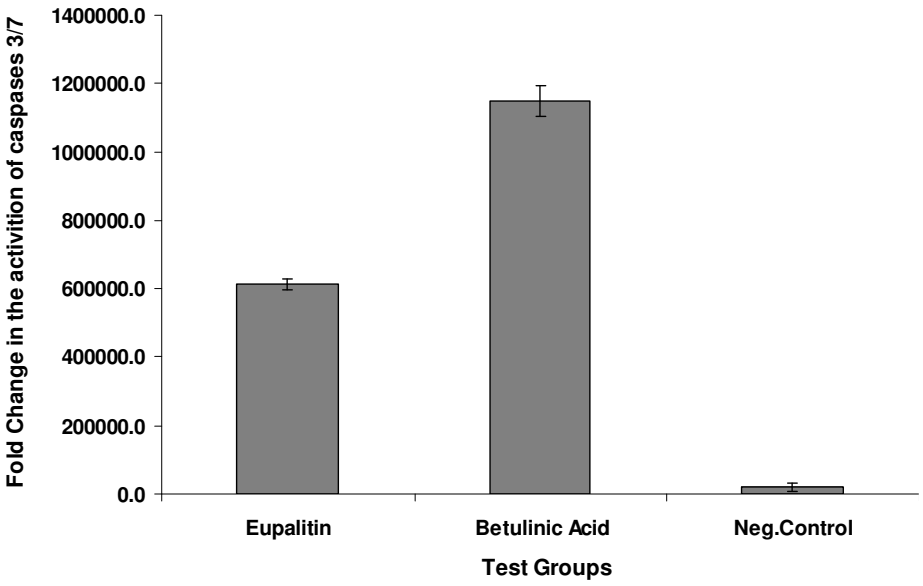

Figure 4. Caspases activity by eupalitin.

REFERENCES

Barron D, Ibrahim RK (1987). 6-Methoxyflavonol 3-monosulphates from *Flaveria chloraefolia*. Phytochemistry 26:2085-2088.

Bold RJ, Termuhlen PM, McConkey DJ (1997). Apoptosis, cancer and cancer therapy. Surg. Oncol. 6:133-142.

DaRocha AB, Lopes RM, Schwartsmann G (2001). Natural products in anticancer therapy. Curr. Opin. Pharmacol. 1:364-369.

Dulabh K, Monga MD, O'Connell MJ (2006). Surgical adjuvant therapy for colorectal cancer: current approaches and future directions. Anal. Surg. Oncol. 13:1021-1034.

Edwards JM, Raffauf RF, LeQuesne PW (1979). Antitumor plants. Part VII. Antineoplastic activity and cytotoxicity of flavones, isoflavones, and flavanones. J. Nat. Prod. 42:85-91.

Ghalib RM, Mehdi SH, Hashim R, Sulaiman O, Valkonen A, Rissanen K, Trifunovic, SR (2010). Isolation and Crystal Structure Determination of 3,5,4' Trihydroxy-6,7-Dimethoxy-Flavone (Eupalitin) from *Asparagus falcatus* (linn). J. Chem. Cryst. 40:510-513.

Hoff PM, Ansari R, Batist G, Cox J, Kocha W, Kuperminc M, Maroun J, Walde D, Weaver C, Harrison E, Burger HU, Osterwalder B, Wong AO, Wong R (2001). Comparison of oral capecitabine vs intravenous fluorouracil plus leucovorin as firstline treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J. Clin. Oncol. 19:2282-2292.

Kalidhar SB (1990). Reassessment of the structures of flavonol glycoside

from *Rudbeckia bicolor*. J. Nat. Prod. 53:1565.
Kumar S (2007). Caspase function in programmed cell death. Cell Death Differ. 14:32-43.
Kupchan SM, Bauerschmidt E (1971). Cytotoxic flavonols from *Baccharis sarothroides*. Phytochemistry 10:664-666.
Lee KH (2004). Current development in the discovery and design of new drug candidates from plant natural product leads. J. Nat. Prod. 67:273-283.
Lowe SW, Lin AW (2000). Apoptosis in cancer. Carcinogenesis 21:485-495.
Martini ND, Katerere DRP, Eloff JN (2004). Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). J. Ethnopharmacol. 93: 207–212.
McCormick S, Bohm B (1986). Methylated flavonoids from *Artemisia lindleyana*. J. Nat. Prod. 49:167-186.
Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65:55-63.
Naik P, Karrim J, Hanahan D (1996). The rise and fall of apoptosis during multistage tumorigenesis: down-modulation contributes to tumor progression fromangiogenic progenitors. Genes Dev. 10:2105-2116.
Pandey R, Maurya R, Singh G, Sathiamoorthy B, Naik S (2005). Immunosuppressive properties of flavonoids isolated from *Boerhaavia diusa* (Linn.). Int. Immunopharmacol. 5:541–553.
Pietras RJ, Weinberg OK (2005). Antiangiogenic steroids in human cancer therapy. eCAM 2:49-57.
Sarin JPS, Singh S, Garg HS, Khanna NM, Dhar MM (1976). A flavonol glycoside with anticancer activity from *Tephrosia candida*. Phytochemistry 15:232-234.
Shao Z, Jia X, Xin R, Jin X, Chen Y (2010). Determination of anticancer activity component of flavonoids from *Hedyotis diffusa* by HPLC. Zhongguo Yaofang 21:1394-1396.
Waddell TG, Elkins SK, Mabry TJ, Norris JA, Ulubelen A (1985). Constituents of *Rudbeckia serotina*. J. Nat. Prod. 48:163-164.
Wei X, Huang H, Wu P, Cao H, Ye W (2004). Phenolic constituents from *Mikania micrantha*. Biochem. Syst. Ecol. 32:1091-1096.
Wen K, Wang C, Yan X, Wu P, Li Z (2010). Research progress of flavonoids biological activity. Caoye Kexue 27(6):115-122.
Zhao X, Zhang Q, Wang Z (2010). A review of the recent study progresses in anticancer activity of flavonoids. Dongbei Nongye Daxue Xuebao 41(4):133-138.