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Full Length Research Paper

The *Cuscuta kotschyana* effects on breast cancer cells line MCF7

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Cuscuta kotschyana **Boiss is classified as a member of Convolvulaceae family. This plant is mainly a central Asian taxon. *Cuscuta* is a parasitic plant that wraps around other plants for nourishment. Previous studies have indicated that *Cuscuta* could be possesses anticancer and immunostimulatory activities. The chemical constituents of *Cuscuta* mainly comprise flavonols like quercetin, kaempferol and other glycosids. These compounds maybe responsible for the biological activities of this plant. In the present study, the effects of flavonoid extracts of *Cuscuta* seed stem and its host plant (vine) was investigated on the growth of breast cancer cell line (MCF-7). The HPLC assay revealed that quercetin is the major compound in the extracts. The MTT assay revealed that after treatment with stem and seed extracts, reduced cell viability in MCF-7 cell line in a dose and time dependent manner. The anti proliferative effect of stem extract was significant from 50 µg/ml, and the inhibitory effect of seed extract was significant at 100 µg/ml, after 48 and 72 h. Annexin assay and flow cytometry analysis also showed these extracts (100 µg/ml seed) and (50 µg/ml stem) induced apoptosis 51 and 34%, respectively in MCF-7 cells after 72 h. These finding suggest that the flavonoid extract of Cuscuta could be useful in breast cancer treatment.**

Key words: Cuscuta, dodder plant, flavonoids, MCF7 cells, apoptosis.

INTRODUCTION

Cuscuta (Dodder) is a genus of about 100 to 170 species of yellow, orange or red (rarely green) parasitic plants. Formerly treated as the only genus in the family Cuscutaceae, recent genetic research by the angiosperm phylogeny group has shown that it is correctly placed in the family Convolvulaceae. The genus is found throughout the temperate to tropical regions of the world, with the greatest species diversity in subtropical and tropical regions; the genus becomes few common in cool temperate climates. *Cuscuta chinensis* Lam, is one of the commonly used herbal constituents in Chinese medicinal tonics to nourish the liver and kidney, in both China and Taiwan. It is often added to porridge and beverages to improve vision and impotence, and also used to prevent abortion as well as aging in clinical treatment (Zheng et al., 1998). Previous studies have indicated that *C. chinensis* possesses anticancer (Nisa et al., 1986; Umehara et al., 2004) and immune stimulatory activities (Bao et al., 2002). In addition, *C. chinensis* glycoside has been demonstrated to exert anti-aging effects and enhance memory by inducing PC12 cell differentiation (Liu et al., 2003). The antioxidant activities and flavonol contents of the water and ethanol extracts from the seed of *Cuscuta kotschyana* Boiss. and of its different organic fractions have not yet been reported. In this study, we used different solvents to extract the seed, stem, and host plant of *C. kotschyana* and investigated their antioxidant and anticancer properties. The NMR assay revealed the most abundance flavones compound at *C.*

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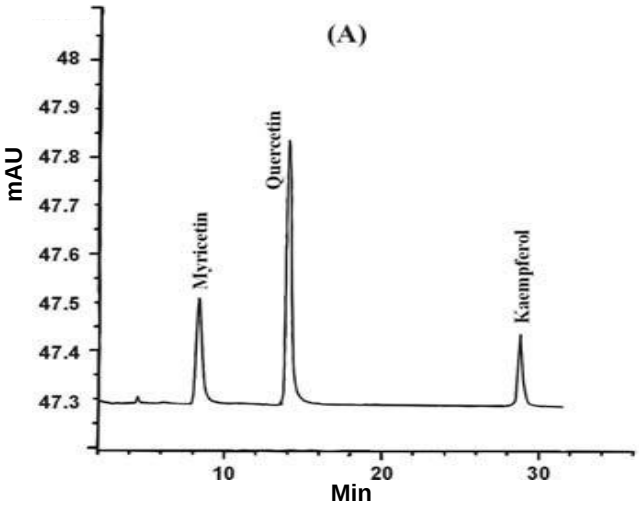

Figure 1. HPLC chromatogram of flavonol aglycon standards (A).

kotschyana.

MATERIALS AND METHODS

Extraction of *C. kotschyana*

The seeds of *C. kotschyana* were collected from rural area of Saveh in Markazi province of Iran authenticated by a Plant Systematic scientist, Dr. Younes Asri, and stored as a voucher specimen (1787) in the Herbarium of Biology Department of Payame Noor University, Najaf Abad, Iran. Different parts (stem, seeds) of *C. kotschyana* and host plant stem (*Vitis vinifera* L.) were powdered and (5 g) decocted, refluxed three times with 50 ml of HCl (2N), and then filtered. The filtrates were extracted by Ethyl acetate 3 times too and then concentrated by rotary vacuum evaporation and then lyophilized with a freeze dryer. The lyophilized powders were collected and stored at room temperature until use.

Determination of the flavonol contents by HPLC

The chromatographic system consisted of Crystal 200 liquid chromatography pump and a Unicame UV detector model 4225. All test samples were separated on a LichroCART_ Purospher_ STAR (250 × 4.6 mm i.d., 5 µm) and the temperature was maintained at an obligatory level of 40°C. The mobile phase was composed of 1% acetic acid in 25 mM phosphate buffer and acetonitrile (50:50), and the pH value was adjusted to 2.5 with phosphoric acid. The flow rate was set at 0.5 ml/min and the wavelength of the detector was kept at 365 nm.

The major constituents in the multiple extracts were identified by comparing their retention time with those of the flavonol standards, such as quercetin and kaempferol 1.0 scan/s (Figure 1). Compounds were identified by computer search using digital libraries of mass spectral data (Liu et al., 2003) and by comparison of their retention indices and authentic mass spectra (Mosmann,1983), relative to C_8-C_{32} n-alkane series (Magee et al., 2006) in a temperature-programmed run.

Cell culture

MCf-7 cells were obtained from Pasteur institute (Tehran, Iran). Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal calf serum, 2 mM glutamine, and 100 µg/ml streptomycin and 100 µ/ml penicillin. Cells were maintained at 37°C with 5% CO_2.

Cell viability

The cell viability was determined using a modified 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay (Mousavi et al., 2009). The MCF-7 cells (were obtained from Pasteur Institute Tehran, Iran) seeded (2000 cell/well) on to flat-bottomed 96-well culture plates. The extracts (25, 50 and 100 µg/ml) dissolved in DMSO (1%) added to each well, incubated for 24, 48; 72 h. Control groups received the same amount of DMSO. Doxorubicin (0.01 to 0.58 g/ml) was used as positive control. After removing medium, *H*-tetrazolium bromide (MTT) solution (5 mg/ml in PBS) was added for 72 h resulting formazan, which was solubilised with DMSO (100 µl) (Mosmann, 1983). The absorption was measured at 570 nm in an ELIZA reader and drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm.

Apoptosis detection

For annexin-based FACS analysis, cells were trypsinized, washed twice in ice-cold PBS, and resuspended in 500 µl binding buffer (Sigma). Annexin V and propidium iodide solution were added to the cell preparations and incubated for 25 min in the dark. Binding buffer (400 µl) was then added to each tube and the samples were analyzed by a FACS Caliber instrument equipped with Cell Quest 3.3 software.

Statistical analyses

All data were presented as percentage compared with vehicle treated control cells, which were arbitrarily assigned 100%. Data were analyzed by one-way analysis of variance followed by Duncan's multiple comparison tests (Sigma Stat, Jandel, and San Rafael, CA, USA). For all comparisons, differences were considered statistically significant at $p < 0.05$.

RESULTS

The effects of quercetin on the viability of human MCF7 breast cancer cells were measured using an MTT assay (Figures 1 to 11). A significant toxicity effect of quercetin appeared after treatment with 50 and 100 µg/ml seed extract for 48 h treatment (Figures 6 and 7) and 100 µg/ml stem extract for 72 h (Figure 10). After a 48 or 72 h exposure, quercetin significantly decreased cell viability in a dose-dependent manner. Compared with the results obtained at 24 h (Figures 2 to 4), significant decrease was observed at 48 and 72 h; however, there was no significant difference between the results obtained at 48 and 72 h. Treatment with high concentrations of quercetin for 72h by stem extraction decreased cell proliferation by up to 67% compared with controls. The seed extract (100

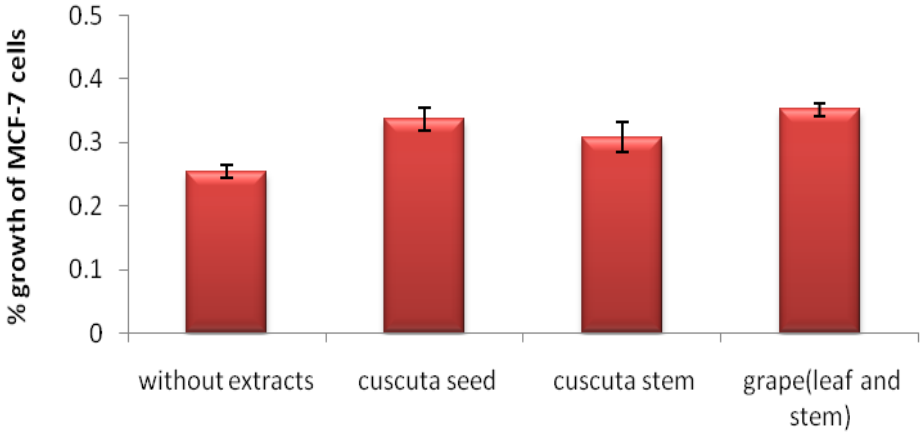

Figure 2. The growth rate of MCF-7 cells at 25 µg quercetin that extracted from different sources after 24 h treatment.

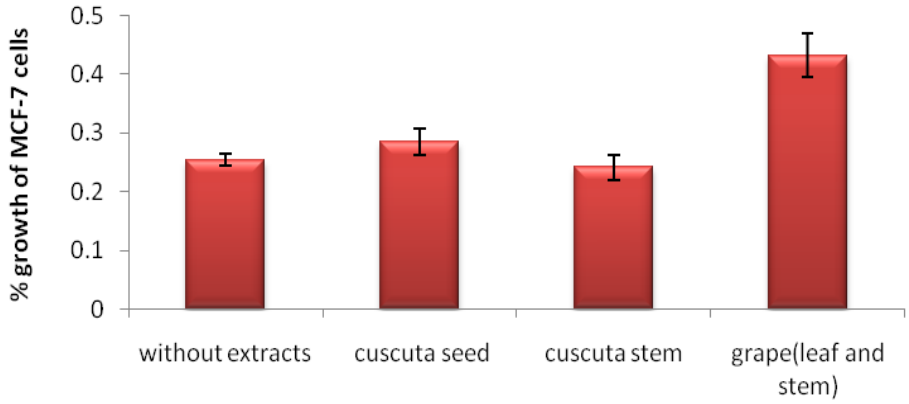

Figure 3. The growth rate of MCF-7 cells at 50 µg quercetin that extracted from different sources after 24 h treatment.

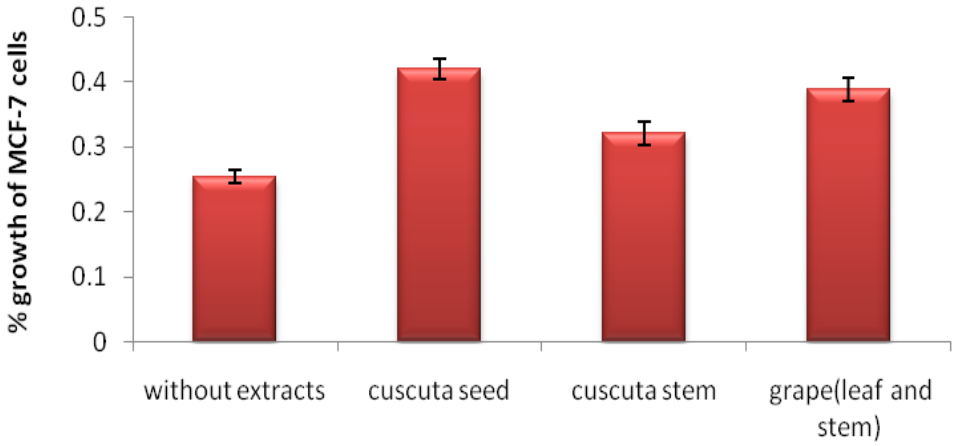

Figure 4. The growth rate of MCF-7 cells at 100 µg quercetin that extracted from different sources after 24 h treatment.

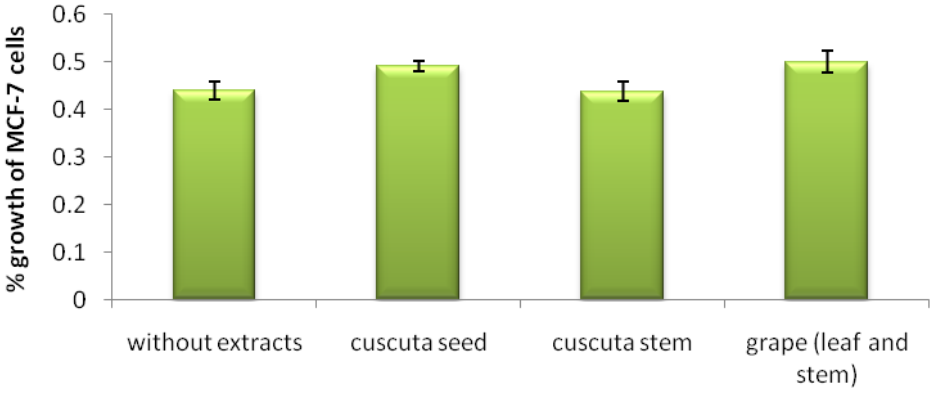

Figure 5. The growth rate of MCF-7 cells at 25 µg quercetin that extracted from different sources after 48 h treatment.

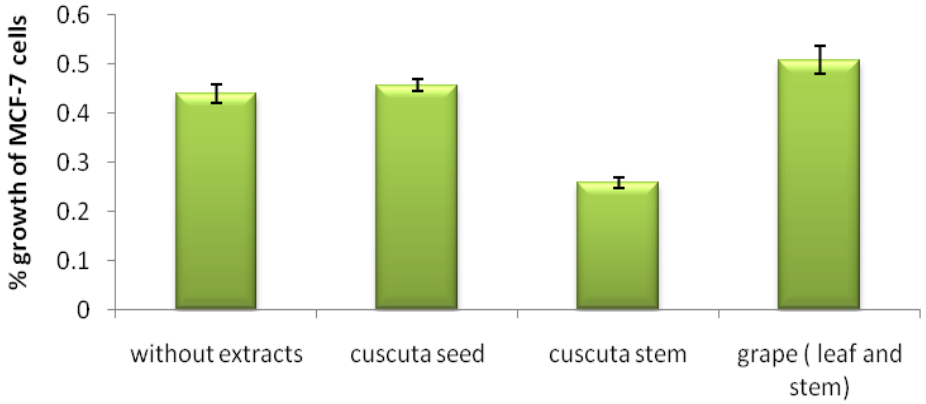

Figure 6. The growth rate of MCF-7 cells at 50 µg quercetin that extracted from different sources after 48 h treatment.

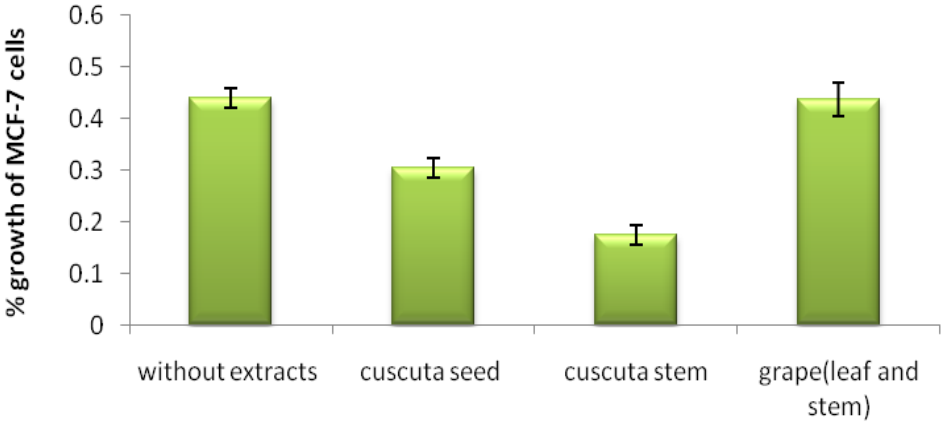

Figure 7. The growth rate of MCF-7 cells at 100 µg quercetin that extracted.

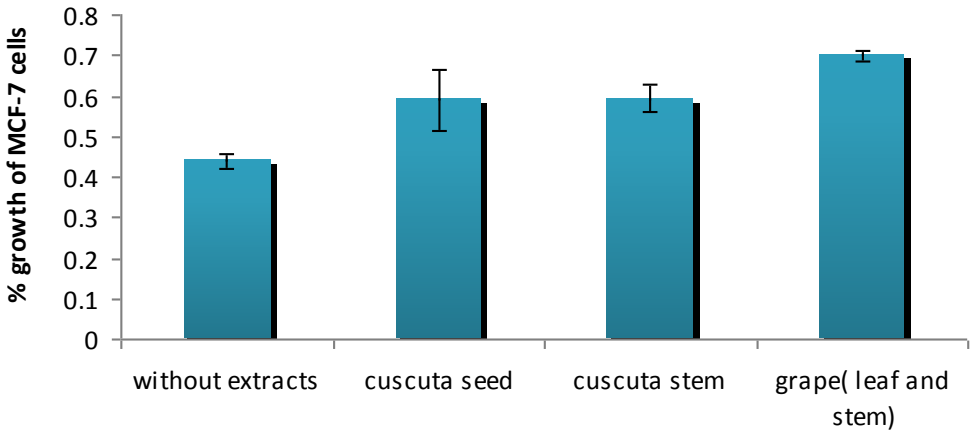

Figure 8. The growth rate of MCF-7 cells at 25 µg quercetin that extracted from different sources after 72 h treatment.

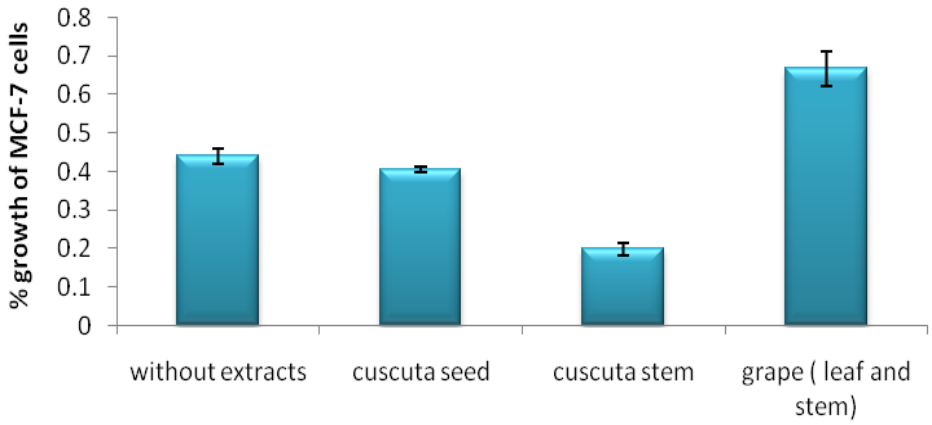

Figure 9. The growth rate of MCF-7 cells at 50 µg quercetin that extracted from different sources after 72 h treatment.

µg/ml) at 48 h had minimum toxicity effect (31%). To investigate apoptosis induction, cells were treated with quercetin at 50 µg/ml stem extract and 100 µg/ml seed extract for 48 h and then subjected to annexin based flowcytometry. Exposure to quercetin significantly affected apoptosis in the MCF7 cells treated with these high concentrations for 48 h. With 50 µg/ml stem quercetin 34% of the cells were shown apoptosis, which is similar to the 51.41% observed with100 µg/ml seed quercetin. In addition, quercetin significantly increased the number of total apoptotic cells in both expose concentrations ($p < 0.05$). Various compounds can induce apoptosis. Owing to their proapoptotic capacity, these compounds are being considered as chemo preventive and therapeutic anticancer agents. In the search for new drugs to treat human breast cancer, we evaluated the proapoptotic activity of *C. kotschyana* extract. As shown in Figure 11, while *C. kotschyana* affected the viability of most of MCF-7 cells, apoptosis only partially contributed in this toxicity. It might be concluded that non-apoptotic cell death to be also involved in *C. kotschyana* induced toxicity in MCF-7 cells.

Cells were exposed to quercetin at high concentrations (50 and 100 µg/ml) and incubated for 48 h. Early apoptotic cells right bottom, late apoptotic cells; right top, live cells; left bottom. *$p < 0.05$, significantly different from the vehicle-only group (0.1% DMSO in medium, that is, quercetin concentration = “0”) (Figure 11).

Quercetin is metabolized from the isoflavone daidzein (Grippo et al., 2007). The cell viability of MCF7 cells was significantly reduced by exposure to various concentrations (25, 50, 100 µg/ml) of extract in a dose-dependent

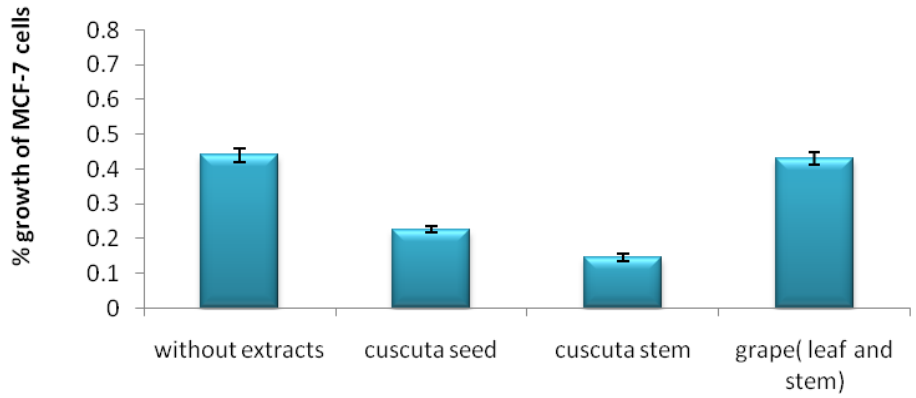

Figure 10. The growth rate of MCF-7 cells at 100 µg Quercetin that extracted from different sources after 72 h treatment.

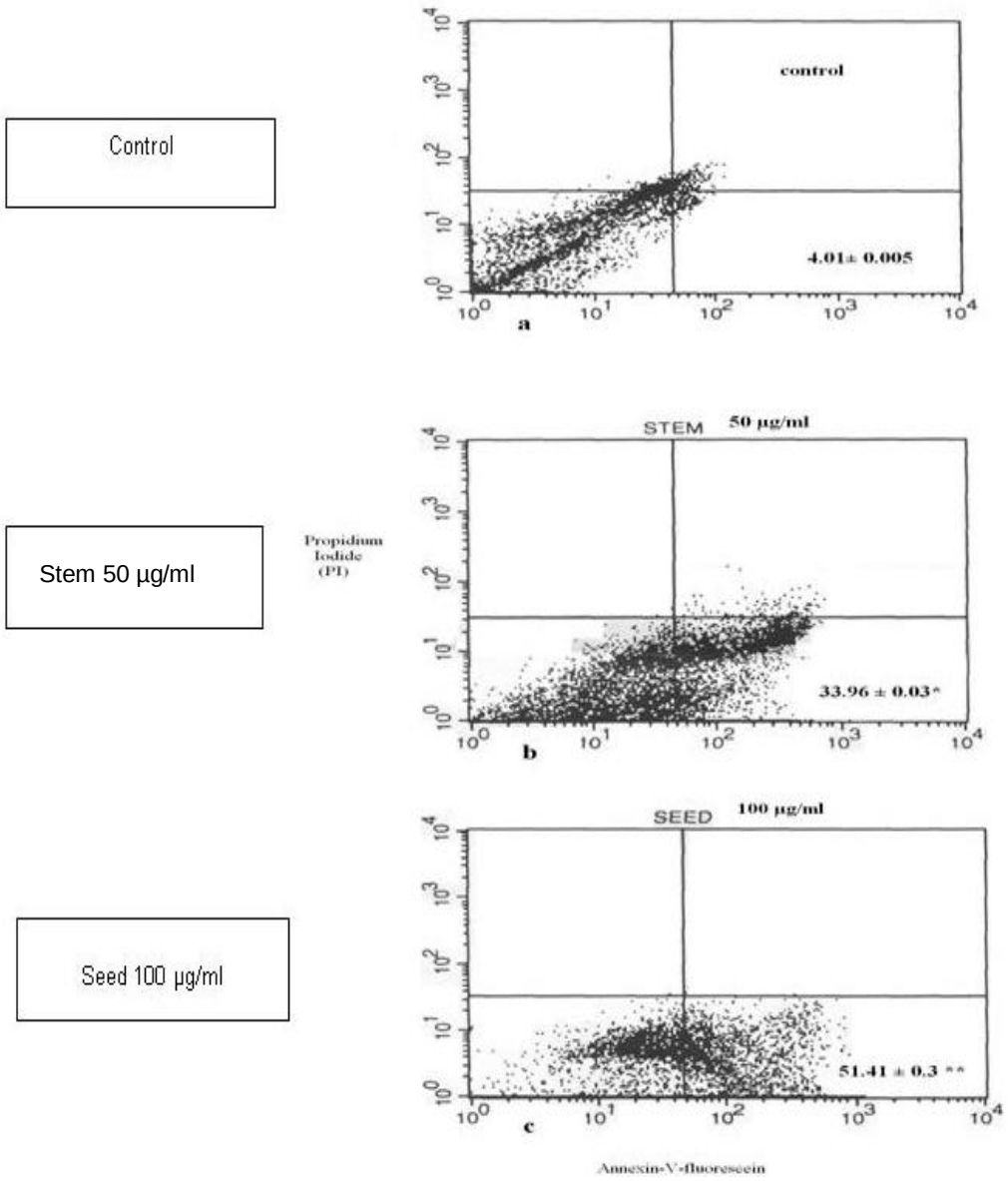

Figure 11. Quercetin -induced apoptosis in MCF7 cells.

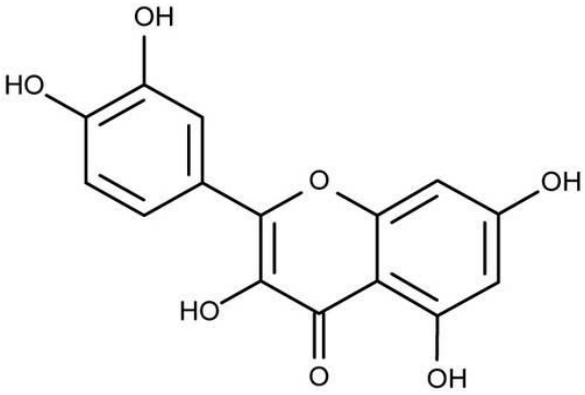

Figure 12. The molecular formula for quercetin is $C_{15}H_{10}O_7$.

manner.

DISCUSSION

Quercetin has been shown to reduced cell viability of several cancer cell lines *in vitro* (Magee et al., 2006; Sathyamoorthy and Wang, 1997). This is consistent with our results showing that the exposure of MCF7 cells to *C. kotschyana* significantly reduced viability. The flavonols, quercetin and kaempferol, are the major active chemical compounds of *C. kotchyana*, and they are also present in many other plants (Szyman´ska and Kruk, 2008). Both quercetin and kaempferol have a 3-hydroxy group of the catechol structure in ring B, and they also have a 2, 3-double bond in conjunction with a 4-carbonyl group in ring C (Figure 12).

The flavonol structure can dramatically increase the resonance stabilization for electron delocalization, as established by its structure – activity relationship (SAR) and this suggests that the SAR of the flavonol structure could enhance the antioxidant and oxidant activities (Han et al., 2009). Concordance between antioxidant activities and flavonol content the results presented have indicated that the antioxidant activities of the seeds from *C. kotchyana* are likely attributed to the flavonol compounds and their quantities in each extract and fraction. The weaker antioxidant activities exhibited by the water extract from the seeds of *C. kotschyana* when compared with its ethanol extract could be explained by the lower contents of these flavonols, due to their poor water solubility (Elisa and Marco, 2005). It has been reported that flavonoids stimulate apoptosis at high concentrations *in vitro* (Haghiac and Walle, 2005). Annexin/PI staining analysis confirmed that treatment with 50 or 100 µg/ml extract for 72 h strongly promoted apoptosis. This is consistent with reports that quercetin is an effective anticancer agent (Choi and Kim, 2008). Many flavonoids significantly reduce cell viability via increase caspase activity. Evidence that flavonoids activate the proteolytic activities of caspases supports their value as potential anticancer agents for controlling tumor growth (Ramos, 2007; Gercel-Taylor et al., 2004; Hedlund et al., 2006; Rassi et al. 2002; Totta et al., 2005). *C. kotschyana* induced apoptosis was involved in induction of cell death. May be the mitochondrial pathway involves release of mitochondrial pro and anti apoptotic proteins (Totta et al., 2005). The significance of non-apoptotic forms of cell death in chemotherapy and the mechanism(s) by which they are induced by hemotherapeutic drugs, remain largely unclear. It is however noteworthy that the non-apoptotic cell death is often observed under conditions in which apoptosis is inhibited. In addition to inducing apoptosis, a number of chemotherapeutic agents have been reported to induce nonapoptotic forms of cell death (Broker et al., 2005). Based on our findings the therapeutic effects of herbal extract should consider in therapy of certain cancer, it seems that the internal pathway mechanisms via mitochondrion are responsible for possible therapeutic effects.

ACKNOWLEDGEMENT

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