International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 2 Issue 1 // January 2013 // PP.23-27

Cytotoxicity of new flavonoid compound isolated from *Farsetia aegyptia*

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ABSTRACT:*Medicinal herbs are also significant source of synthetic and herbal drugs. So far, pharmaceutical companies have screened more than 25,000 plants for anti-cancer drugs. So we done to discover the anticancer activity of the phenolic compound isolated from terrestrial Egyptian plants, the EtOH extract of the aerial parts of the Egyptian medicinal plant farsetia aegyptia (Forssk.) Boiss. was in vitro investigated for cytotoxicity against HCT116, HepG2 and MCF-7 cell lines, and resulted with IC_{50} = 4.65, 12.60 and 17.90 µg/ml, respectively. Doxorubicin (+ve control) showed in vitro cytotoxic activity with IC_{50} = 3.64, 4.57 and 2.97 µg/ml, respectively, The phenolic-rich fraction of the EtOH extract was subjected to further fractionation, which led to the isolation of new flavonoid (kampferol 7-8 diglucoside); low polar fractions revealed the isolation of other known compound which identified, according to its' spectral data and comparison with the literature, Furthermore new flavonoid compound give high cytotoxicty against Hela and MCF-7 cell lines,also cytotoxity of the isolated known compounds were studied*

Keywords*: crucifereae ,Farsetia aegyptia, flavonoid glucoside ,anticancer*

I. INTRODUCTION

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today we still rely largely on the curative properties of plants.

According to World Health Organization, 80 % of the people living in rural areas depend on medicinal herb as primary healthcare system , The herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body (Larkin 1983), (Saxe 1987).

Herbal medicines have a vital role in the prevention and treatment of cancer. With advanced knowledge of molecular science and refinement in isolation and structure elucidation techniques, various anticancer herbs has been identified, which execute their therapeutic effect by inhibiting cancer-activating enzymes and hormones, stimulating DNA repair mechanism, promoting production of protective enzymes, inducing antioxidant action and enhancing immunity of the body (Sakarkar and. Deshmukh 2011) . *Farsetia aegyptia* is medicinal plant belong to family Cruciferae, are known to be used by the native Bedouins as an ant diabetic and antispasmodic and it used for relive of rheumatic pains and taking as a cooling medicine after pounding (Mitchell-Olds et al., 2005). Family Cruciferae is one of the largest families in the plant kingdom which is rich in medicinal plants. It includes 338 genera and 3350 species that are distributed worldwide , it is represented in Egypt by 53 genera and 107 species (Tackolm 1974) , (Boulos 1999). The plants of this family are used as antidiabetic, antibacterial, antifungal, anticancer, antirheumatic and show a potent insecticidal effect (Rizk 1986).

Many of the cruciferous plants serve as a source of food and condiment such as cabbage, turnip, radish and mustard. All of them contain unusual flavonol glycosides and glucosinolates. The latter upon hydrolysis provides many of the culinary flavors characteristic to these plants(Tooky 1980)

The present work described isolation phenolic and triterpenoid compounds from *Farsetia aegyptia* and study their cytotoxic activity. *Farsetia aegyptia* grows naturally in North Sinai, it has medicinal used and there are little studies concern with the isolation of active compound. **(Shahat et al., 2005)** isolated three flavonol di-O-glycosides compound from butanol extract using (LC/UV-DAD) .

II. MATERIALS AND METHODS

2.1. Plant material

The leaves of *Farsetia aegyptia*, was collected from South Sinai during spring season at 2009, The plant samples were kindly identified by Dr/ Ahmed Morsy Professor in Botany department, Desert Research

Center. A voucher specimen of the plant materials were kept in the Herbarium of Desert Research Center. No drcc20/844. The plant material was air dried in shade and grinded to fine powder for phytochemical and biological investigation.

*F. **aegyptia*** is a large herb, 20-40 cm tall, the leaves are large (0.5-1 m long), alternate, pinnately compound with 10-15 pairs of opposite leaflets, 5-20 cm long. The flowers are small and yellow-green with a garlic odor. The fruit is a five-valves woody capsule, 6 cm long that opens to release numerous flat-winged seeds this descriptions of the plant as (Tackolm 1974) , (Boulos 1999).

2.2General experimental conditions

Thin layer chromatography (TLC) (Silica gel G-60 F_{254} Merck, Germay). Column chromatography (Silica gel G-60, 70-230 mesh). Paper chromatography (Whatman No. 3). Nuclear Magnetic Resonance (NMR) spectra were measured at 600.17 and 150.91 MHz for 1H- and 13C-NMR respectively, with a JEOL ECA 600 spectrometer. Solvent systems; I- chloroform -methanol (9:1), II- ethyl acetate – methanol – water (30:5:4), III- ethyl acetate – methanol – acetic acid – water (65:15:10:10) and IV- butanol– acetic acid – water (4:1:5 upper layer) were used.

Visualization of chromatograms was achieved under UV (254 and 365 nm) before and after exposure to ammonia vapor or by spraying with aluminum chloride for flavonoid and spraying by vanillin sulphoric acid for terpene.

2.3 **Extraction and isolation**

The air-dried powder of investigated plants **(1 kg)** was extracted by percolation in (70 %) ethanol and filtered off. The extraction repeated four times. The combined ethanol extracts were concentrated under reduced pressure at temperature not exceeding 40^{o}C till dryness., concentrated in rotary evaporator till dryness and then dissolved in small amount of methanol with stirring to remove salts; concentrated till dryness. The dried extract was fractionated by dissolving in petroleum ether, ethyl acetate and n-butanol by separating funnel. Each extract was dried over anhydrous sodium sulphate and concentrated again as before.

Ethyl acetate extract (3.5 g) was subjected to flash liquid chromatography (FLC) and eluted with 100% chloroform and ethyl acetate. Fractions (200 ml each) were collected. The collected fractions were obtained according to TLC manner using system ethyl acetate: methanol: water (30:5:4). Three fractions **(A, B, C)** were obtained containing flavonoid and terpenoid derivatives.

Fraction **A** (1.0g) was applied to preparative TLC using 15 plates (20×20 cm) by system (c). Two major bands were visualized under UV lamp, collected, scratched and eluted by methanol: water (9:1 v/v) several times. Every band was dried under reduced pressure, separately introduced to column sephadex LH-20 using eluting system methanol/water. Two pure compounds were obtained when chromatographed to (TLC) each gave a single fluorescence spot. These two compounds were recrystallized from methanole give , A1(1.5g) was separated by silica gel column by system (c) to give compound **F_1 Kaempferol** and **F2** Qurectin which identified by comparison with literature (Mabry, et al., 1970), (Markham 1982).

Fraction **C** (1.9g) was purified on sephadex LH-20 and the eluting system was methanol/water. The obtained fractions were subjected to thin layer chromatography (TLC) that gave a single spot indicating one compound which was recrystallized from methanol giving compound **F_3.** Two dimension paper chromatography of this compound using system BAW (4:1: 5) and AcOH-15% revealed the presence of one spot of flavonoid nature.

Compound **F_3** (40 mg) was obtained as yellowish brown powder, mp.226-27 □C. Structure of compound **F_3(new compound kamperol 7,8** diglucoside) was confirmed by physical methods including U.V, ^{1}H, ^{13}C ,H- NMR and LC/MS

Where fractions **(B** was subjected to column chromatography) and eluted with 100% hexane and ethyl acetate. Fractions (200 ml each) were collected. The collected fractions were obtained according to TLC manner using system ethyl acetate: methanol: water (30:5:40), containing ,known compounds betulin, Friedelin, coumarin , Scopltine and B-amyrin(Awaad et al 2001), (Rzeski, et al 2009), which identified by authentic sample and comparison HNMR with literature.

2.4Cytotoxicty assay procedures

Human tumor cell lines

non small cell lung adenocarcinoma (A549), human colon carcinoma (HCT116), epitheloid cervix carcinoma (Hela), human hepatocellular liver carcinoma (HepG2), normal melanocytes (HFB-4), human breast carcinoma (MCF-7) cell lines were obtained in frozen state under liquid nitrogen (-180ºC) from the American Type Culture Collection. The tumor cell lines were maintained by serial sub-culturing in the National Cancer Institute, Cairo, Egypt.

Culture media

The cells were suspended in RPMI 1640 medium (SIGMA ALORICH) supplemented with 10% fetal calf serum (SIGMA, USA) in presence 1% antibioticantimycotic mixture (10.000 U/ml K-penicillin, 10.000 µg/ml streptomycin sulphate and 25 µg/ml amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

Assay method for cytotoxic activity

The cytotoxicity against A549, HCT116, Hela, Hep-G2, HFB4 and MCF-7 cells were tested in the National Cancer Institute, according to the SRB (Sulforhodamine B) assay using MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) method by (. Skehan et al 1990), Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug. Briefly, cells were seeded in 96-multiwell plates at densities of 5×10^4–10^5 cells/well in a fresh media and incubated under normal growth condition for approximately 24 h before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 200 µl aliquot of serial dilution with DMSO (100%) of alcoholic extract and isolated compound (5.0, 12.5, 25, 50, 100 µg/ml) were added and the plates were incubated for 24, 48 and 72 h at 37°C in a humidified incubator containing 5% CO2 in air.

Control cells were treated with vehicle alone. Four wells were prepared for each individual dose. Following 24, 48 and 72 h treatment, cells were fixed, washed and stained with Sulforhodamine B stain (Sigma, USA). Colour intensity was measured in an ELISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany).

Statistical analysis

All values were expressed as the mean of percentage of inhibition cells of the four replicates for each treatment. Data were subjected to SPSS (ver.8.0). $P<0.05$ was regarded as significant.

III. RESULT AND DISCUSSION

Methanol extract of *Farsetia aegyptia* revealed the isolation of three compounds, these compounds were, quercetin , kampferol and new compound **Kaempferol-7, 8- ( ) diglycoside**

These compounds F3, yellowish brown powder , mp.226-27 C , R_f-values (0.56),UV spectral data of compound $\mathbf{F_3}$ in MeOH showed two absorption bands at λ_{max} 271, 356nm for band II and I respectively. This indicates that it is flavonol with free OH at position 3. On addition of NaOMe it exhibited a bathochrmic shift in band I (+40nm), indicating a free OH group at position 4'. No shift in band II on the addition of NaOAc indicating that C-7 position is occupied. Addition of boric acid gave no shift indicating the absence of orthodihydroxy group at B-ring. On addition of $AlCl_3$ it exhibited a bathochrmic shift in band I (+37nm), indicating the presence of

On addition of $AlCl_3$ it exhibited a bathochrmic shift in band I (+37nm), indicating the presence of a free OH group at C-5 only and this shift was not affected after addition of HCl indicating the absence of orthodihydroxy group at B-ring. All of these indicate that it is flavonol with substitution at C7

^{1}H-NMR . spectrum of this compound $\mathbf{F_3}$ in $DMSO_{d6}$ showed four protons of B-ring as H-2' proton and H-6' proton appeared (at δ 7.3ppm) as doublet of doublet with J = 8Hz due to H-2' and H-6' are superimposed. H-3' proton and H-5' proton appeared (at δ 6.8ppm) as doublet of doublet with J = 8Hz due to H-2' and H-6' are superimposed. H-6 in A-ring show down field (at δ 6.4, ppm) as a result of substitution at position 7. The lack of H-8 proton indicates the compound F3is flavonol C- glucoside Marie-Aleth et al., 1984)**.**The lack of H-3 proton of C-ring indicates that C-3 is hydroxyl group (OH) free or substituted. The ^{1}H-NMR spectrum revealed the signal of anomeric proton of glucose as doublet (at δ 5.4ppm) with J = 7.3Hz, signal of anomeric proton of C- glucoside as doublet (at δ 4.52ppm) with J = 9.6Hz, signals for remaining sugar protons are evidant at 3.0-4.0ppm as multiplet .

^{13}C-NMR spectrum of compound $\mathbf{F_3}$ in $DMSO_{d6}$ showed the following signals: 179.7 of the highest oxygenated carbon ketonic carbon at C-4, 161.5 for C-7; 115.7 for C-3`; 154.48 for C-4`; 132.08 for C-3; 128.3 for C-6`; 115.76 for C-5`; 128.3 for C-2`,Two anomeric protons appeared at 101.8, 102.1 for C-1`` and C-1```,The lower affected aromatic carbons C-6 and C-8 appeared at 91 and 84.8 respectively, The Sugar carbon carbons appeared at 65to 76.3

L-C Mass spectrum of compound $\mathbf{F_3}$ showed peak at (M-1) 609 and other important peak at M 519, 489 and 327.**Acid Hydrolysis,** of compound $\mathbf{F_3}$ by 0.1 N HCl (controlled or mild acid hydrolysis) afforded kaempferol as the aglycone. The sugar moiety was identified as glucose with authentic pure sugars on paper chromatography (PC).

From the above mentioned data and by comparing with the published data compound $\mathbf{F_3}$ was confirmed to be identified as **Kaempferol-7, 8- ( ) diglycoside** By searching with the published data the compound $\mathbf{F_3}$ wasn't published pervious it is was studied for the first time

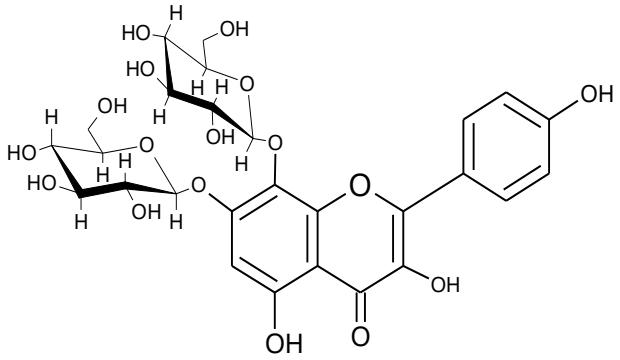

Figure 1. Structures of new compound

Table (1): Cytotoxic activity of ethanol extract of *Farsetia aegyptia* (Forssk.) Boiss. against cultured different cell lines *in vitro*

Human Cell line / Conc. Ppm	% of Inhibition			
	100	50	25	12.5
A549	42.3±0.23*	64.84±0.17*	59.09±0.29*	5.38±0.06*
HCT116	-40.50±1.15*	-25.59±1.73*	0.0±	-
HepG 2	39.1±0.46*	25.00±0.06*	16.78±0.64*	0.0±
MCF7	13.1±0.23*	5.43±0.12*	0.0±	-

Each value represents the inhibition growth related to Doxorubicin.
Activity □ 75%: high, 75-50% :good, 50-25 % : normal and □25%: weak activity.
P□0.005

Table (2): Cytotoxicity of Isolated compounds against cultured A549 (lung human cell line) *in vitro*:

Sample / Conc. Ppm	**% of Inhibition ± SEM**			
	100	**50**	**25**	**12.5**
Betulin	**43.7±1.05***	**41.92±0.75***	**15.72±0.81***	**0±0.005**
Friedelen	**34.9±0.31***	**39.52±1.45***	**35.85±1.07***	**0±0.02**
□ –Amyrin	**- 43±2.5***	**-48.75±2.48***	**0±0.003**	**-**
Scopltine	**-34±1.33***	**-11.57±3.17**	**0±0.002**	**-**
coumarin (44)	**-15.3±1.45***	**0±0.32**	**-**	**-**

Each value represents the inhibition growth related to Doxorubicin ±SEM (Standard Error of Mean).
*Significantly different from Doxorubicin value at P□0.005 according to paired-samples *t*-test
Activity □ 75%: high, 75-50% :good, 50-25 % : normal and □25%: weak activity.

Table (3): Cytotoxicity of new compound against cultured different human cell line *in vitro*

Human Cell line / Conc. Ppm	**% of Inhibition ± SEM**				
	50	**25**	**12.5**	**5**	**IC50**
HFB-4	**79.22±0.02**	**44.16±0.14**	**19.10±0.03***	**0±0.05***	**32.1**
HCT116	**90.05±0.03**	**79.55±0.02**	**55.45±0.01***	**25.63±0.01***	**13.5**
HepG 2	**80.52±0.02**	**106.83±0.01**	**52.80±0.06***	**2.30±0.06***	**16.1**
MCF7	**102.7±0.001**	**110.10±0.0003**	**60.30±0.006***	**42.04±0.01***	**12.6**
Hela	**97.45±0.01**	**94.69±0.01***	**60.69±0.06***	**6.65±0.05***	**12.7**

Each value represents the inhibition growth related to Doxorubicin ±SEM (Standard Error of Mean).
*Significantly different from Doxorubicin value at P□0.005 according to paired-samples *t*-test
Activity □ 75%: high, 75-50% :good, 50-25 % : normal and □25%: weak activity.

The cytotoxic activity of crude extract of *farsetia aegyptia* was *in vitro* assessed against A549, HCT116, HepG2 and MCF7. The percentages of inhibition related to the reference drug (doxorubicin) are given in Table (1). The crude extract at concentration 50 and 25 µg/ml showed good cytotoxicity against A549, while the concentration at 100 µg/ml against A549 and 100 and 50 µg/ml against HepG2 showed normal activity when compared with doxorubicin. The isolated compound were evaluated against A549 *in vitro*. The

cytoxic activity of triterpene butelin and fridelin related to doxorubicin exhibited normal cytotoxic activity. However, the compound Kaempferol-7, 8-diglucoside was evaluated its cytotoxic activity against HCT116, HepG2, Hela, HFB4 and MCF7 and the percentages inhibition related to the reference drug (doxorubicin) were presented in Table (3).

Cytotoxicity of isolated compounds against cultured A549 (lung human cell line) was studied *in vitro* as in table (2)most compound show normal activity especially betulin this agree with these reference(Wada et al., 2001) who says Triterpenoids have been reported to act as selective catalytic inhibitors of human DNA topoisomerases which it play important roles in replication, transcription, recombination and chromosome segregation at mitosis(Beretta et al., 2008). Betulin elicits anticancer effects in tumor primary cultures and cell lines in vitro whereas, it exhibited anti proliferative effect, altered tumor cells morphology, decreased their motility and induced apoptotic cell death (Rzeski, et al., 2009) On the other hand, have been reported that β-amyrin exhibited weak cytotoxicity against A549 and HL-60 cancer cell lines. (Thao et al., 2010)

IV. CONCLUSION

The plant *farsetia aegyptia* for the first time improve it have Cytotoxic activityof EtOH extract of the aerial parts of the plant was *in vitro* investigated against HCT116, HepG2 and MCF-7 cell lines, and resulted with IC_{50} = 4.65, 12.60 and 17.90 µg/ml, respectively. This may be due to the presence of flavonoid compound especially flavonoid glycoside(new compound,kampferol 7-8 diglucoside) which also show high cytotoxic ty which isolated from high polar fraction of the EtOH extract, low polar fractions revealed the isolation of other known compound which identified, according to its' spectral data and comparison with the literature, terpene compounds(betulin, β-amyrin and Friedeline), coumarin and scopletin also show normal activity against lung human cell line .there are more studies on isolation of flavonoid compound from *farsetia aegyptia* but in our study we isolate new compound and study the cytotoxicity of this compound.

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