

Original Research Article

Phytochemicals Analysis and Biological Activity For The Ethanolic Extract of *Punica granatum* Rind

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Abstract

The present work was pointed the recognizable proof of the presence of phytochemicals in ethanolic concentrate of nearby *Punica granatum* rind by utilizing gas chromatography/mass spectroscopy; an in vitro investigation of the impact of ethanolic unrefined concentrates of *Punica granatum* rind on cervical tumor human cell lines (Hela) after 24hour of exposure. The ethanolic concentrate was set up from dried skin of *Punica granatum*. Yield of concentrate was 12.3%. The ethanolic extract exhibit dose-dependent, cell specific inhibitory effects on human cervical cancer cell line (Hela), was exponentially inhibited with increasing concentration of each extract. Ethanolic extract was produced a clear significant inhibition on Hela cell line, with IC₅₀ values equal to 143.5µg/dl.

Key Words: *Punica granatum*, Hela cell line, Phytoanalysis, Biological activity, GC/mass spectroscopy.

الخلاصة

كان هدف البحث الحالي هو تشخيص المركبات الموجودة في المستخلص الكحولي لقشور الرمان المحلي باستخدام جهاز كروماتوغرافيا الغاز/مطياف الكتلة، فضلا عن دراسة تأثير المستخلص الايثانولي لقشور الرمان على الخط الخلوي لسرطان عنق الرحم. وكانت حصيللة الاستخلاص هي 12.3 %، ان المستخلص الايثانولي لقشور الرمان اظهر تأثيرا تثبيطيا معتمدا على الجرعة تجاه الخط الخلوي لخلايا سرطان عنق الرحم ، وازداد التثبيط بزيادة التركيز ، وقد كانت قيمة التركيز المثبط ل50% من الخلايا هو 143.5مايكروغرام/مل.

Introduction

Herb constituent a critical wellsprings of dynamic normal mixes which vary broadly in term of natural movement and component of activity. *Punica granatum* is a little tree local to Mediterranean area and has been utilized broadly as a part of the type of juice condensed, canned drink, stick and jam [1]. It is additionally utilized therapeutically as a part of Europe, India, china, Bedouin country. The plant utilized as a part of society medication for the treatment of different maladies, for example, ulcer, hepatic harm, and snakebite [2]. The skin of

natural product is utilized as anthelmintic, helpful in diarrhea and ulcer [3]. The entire plant, however specifically the recreation center, is antibacterial, antiviral and astringent. A decoction of seeds is utilized to treat syphilis and juice is utilized to treat jaundice and looseness of the bowels [4]. Test examines have exhibited its pain relieving, anthelmintic, antibacterial, antidiarrheal, antifertility, antifungal, mitigating, antimutagenic, antispasmodic, and hypoglycemic action [5-8]. In the respect of the above data, the present work was done to appraisal the phytochemicals by GC/MS, and to assess

the cytotoxic action of ethanolic concentrate of *Punica granatum* rind.

Materials and Methods

The *Punica granatum* dried rind were gotten from market in Iraq. The fruits was peeled and the peel was shaded and dried and powdered with manual processor. Roughly 100gm of dried powdered of skin was macerated in 500ml of 99% ethanol for around 72h with shaker water shower at 45c. The concentrate was sifted through bandage and afterward thought to sloppy encourage by vanishing at 45c in hatchery. The concentrate was kept in cooler until utilized. Further examinations including phytochemical investigation and cytotoxic exercises were done on rough concentrate .

GC/Mass Spectroscopy Analysis

GC-MS examination of this concentrate was performed utilizing GC SHIMDZU QP2010 framework and gas chromatograph interfaced to a mass spectrometer (GC-MS) furnished with Tip top melded silica slender section. The relative rate measure of every segment was figured by contrasting its normal top territory with the aggregate range. Programming received to handle mass spectra and chromatograms was a GC-MS solution 1875.

Cytotoxic Effect Of *P. granatum* Dried Rind

As indicated by Freshney [9], subculturing for Hella cell line was done at the purpose of monolayer.

Six groupings of dried concentrate of *Punica granatum* extract were readied utilizing serum free media (12.2, 25, 50, 100, 200, 400 µg/ml).

Treatment Of Hella Cell Line With The Extract

At the point when the Hella cell line was at log stage after 24h of hatching, the impacts of *Punica granatum* skin concentrate was considered by Betancur-Galvis [10]. MTT color was utilized to ponder the cytotoxic impact of concentrate on Hella cell line, the optical density at 492nm was measured utilizing Eliza spectrophotometer .

The inhibition rate was ascertained by Goa, et al. (2003):

$$IR = (O.D \text{ of control} - O.D \text{ of test}) / O.D. \text{ of control} \times 100$$

Inhibitory concentration of 50% of cells (IC₅₀), was calculated using Graph Pad Prism v.6.

Results and Discussion

Ethanolic concentrate of *Punica granatum* rind was readied, these concentrate gave 12.3gm of sticky yellow 12.3% yield

The ethanolic concentrate of *Punica granatum* rind yielded fifteen compounds were appeared by GC/MS examination (Table1), and the most plenteous mixes were 2-furancarboxyaldehyde, 5-(hydroxymethyl)- (79.68%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.34%), and furfural (4.31%). Different parts distinguished by GC/MS were in less degree.

Table 1: Phytocomponents identified in the ethanolic extract of *Punica granatum* rind

Peak	RT	Area	Area %	Name of compound
1	8.69	1205261	4.31	Furfural
2	9.13	430203	1.54	Formic acid
3	9.42	311684	1.11	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	10.04	229439	0.82	2-furancarboxyaldehyde,5-methyle-
5	10.49	152339	0.54	Benzenamine,N,N,4-trimethyle-
6	11.11	329606	1.18	2-furanmethanol
7	11.84	454197	1.62	2,5-furandione,3-methyle-
8	12.52	69896	0.25	2(5H)-furanone
9	12.68	101111	0.36	Glutamine,N-methyle-
10	13.95	215108	0.77	N-(3-methyle-2,5-dioxo-imidazolidin-4-yl)-acetamide
11	15.76	263851	0.94	3-furancarboxylic acid, methyl ester
12	17.61	241040	0.86	Methyl-2-methoxy-4-methylpent-2-enoate
13	18.43	1492953	5.34	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyle-
14	18.95	186332	0.67	3-hexanone,2,5-dimethyl-4-nitro-
15	20.50	22290970	79.68	2-furancarboxyaldehyde,5-(hydroxymethyl)-
		27973990	100.00	

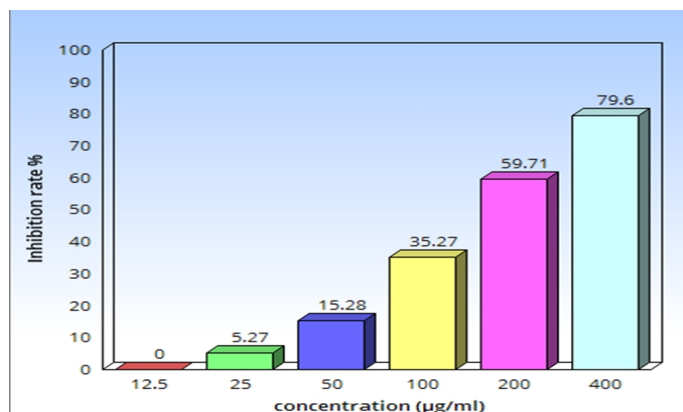
Cytotoxic effect of *P. granatum* rind extract on Hella cell line:

The outcomes in the table (2) uncovered that ethanolic concentrate of *P. granatum*

has a noteworthy inhibitory impact on Hella cell line after 24h of presentation.

Table 2: Inhibition rate of ethanolic extract of *punica granatum* on Hella cell line after 24h. of exposure

Con. (µg/ml)	Inhibition rate ±SE	Initial significant. Con.	C.S
12.5	0	25 µg/ml $P\text{-value} \leq 0.000001$	One-way ANOVA $P=0.775$ $\text{Chi-square}=1.784$ $d.f=4$
25	5.27±0.091		
50	15.28± 0.13		
100	35.27±0.077		
200	59.71±0.14		
400	79.6± 0.192		

**Figure2:** Inhibition rate of ethanolic extract of *Punica granatum* rind on Hella cell line

The initial significant concentration was 25 μ g/ml at profoundly huge contrast ($p \leq 0.000001$), moreover the bit by bit expanding in the convergence of ethanolic concentrate of *P. granatum* was directed to increase the inhibitory rate of

Hella cell line, regardless of no critical distinction between the concentrations (25-400 μ g/ml) individually at ($P=0.775$). The IC_{50} started at 143.5 μ g/ml reaching to 400 μ g/ml, figure (3).

$IC_{50} = 143.5$

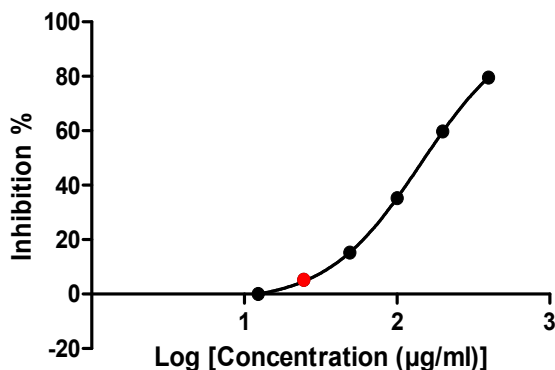


Figure3: IC_{50} measurement using Graph Pad Prism v.6

Late studies uncovered that the tannins a polyphenolic nature (the fundamental constituents of *P. granatum* skin) structure buildings with proteins, sugars, gelatin and alkaloids. It has cancer prevention agent and antibacterial impact [11]. The hydrolysable tannin was appeared to display moderate cytotoxicity against refined human tumor cell lines including A549, SK-OV-3, HT-1080, K562 and S180 in vitro [12]. The Aggregate *P. granatum* tannin concentrate was assessed for hostile to proliferative movement in vitro on human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620) and prostate (RWPE-1, 22Rv1) tumor cells and apoptotic impacts were assessed against the HT-29 and HCT116 colon disease cell lines. They were appeared to actuate apoptosis and diminished the practical cell number of human oral, prostate and colon tumor cells [13]. It was resolved that tannins were displayed hostile to tumor and anticancer movement against to HeLa cell and murine leukemia cells (L1210/0), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (Molt4/C8, CEM/0) [14].

As indicated by a work distributed in the 2010 year; tannic corrosive were kept the actuation of PARP-1, diminished Bax and expanded Bcl-2 expression in H9c2 cells, in this way, forestalling doxorubicin-impelled cell passing [15].

Specialists demonstrated that tannic corrosive TA-incited apoptotic passing in intense myeloid leukemia (AML) HL-60 cells by means of measurement and time-subordinate way and also increment of sub-G1 division, chromosome buildup and DNA discontinuity [16].

Xiong Y and companions investigated that the defensive impacts of tannins in *Sanguisorba radix* (Rosaceae) (TSR) on myelo concealment mice actuated by cyclophosphamide (CTX). Subsequently, TSR could fundamentally build the quantities of white platelets, red platelets and platelets of myeloid concealment in mice. Besides, it could quicken bone marrow hematopoietic stem/ancestor cells (HSPCs) in myeloid concealment mice and improve cell multiplication by advancing cell cycles from G0/G1 stage to access into S and G2/M stages [17]. Apoptotic action is expanded in breastcancer and prostate tumor

cells in light of introduction to tannin removes [18].

Conclusion

The present work demonstrated that *Punica granatum* rind comprise of various phytochemicals, besides the ethanolic concentrate of *P. granatum* rind cytotoxically affect cervical cell carcinoma utilizing Hella cell line as a model of study.

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