Original Research Article

Phytocomponents 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 phytocomponents 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 24-hour 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.

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 anthelminthic, 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, anthelminthic, 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 phytocomponents 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 phyto-chemical 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 solution1875.

**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 of control-O.D of test)/ O.D. of control × 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: Phytochemicals identified in the ethanolic extract of *Punica granatum* rind

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

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

<table>
<thead>
<tr>
<th>Con. (µg/ml)</th>
<th>Inhibition rate ±SE</th>
<th>Initial significant. Con.</th>
<th>C.S</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>0</td>
<td>25 µg/ml</td>
<td>One-way ANOVA</td>
</tr>
<tr>
<td>25</td>
<td>5.27±0.091</td>
<td><em>P</em>-value ≤ 0.000001</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>15.28±0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>35.27±0.077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>59.71±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>79.6±0.192</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Inhibition rate of ethanolic extract of *Punica granatum* rind on Hella cell line
The initial significant concentration was 25µg/ml at profoundly huge contrast (p≤0.000001), moreover the bit by bit expanding in the convergence of ethanolic concentrate of *P. granatum* rind was directed to increase the inhibitory rate of Hella cell line, regardless of no critical distinction between the concentrations (25-400µg/ml) individually at (P=0.775). The *IC50* started at 143.5µg/ml reaching to 400µg/ml, figure (3).

\[
IC50 = 143.5
\]

![Inhibition percentage vs concentration](image)

**Figure3:** IC50 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 phytocomponents, besides the ethanolic concentrate of *P. granatum* rind cyto-toxically affect cervical cell carcinoma utilizing Hella cell line as a model of study.

**Acknowledgment**

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**References**


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