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# Molecular docking prediction and in vitro studies elucidate anti-cancer activity of phytoestrogens

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ABSTRACT

*Aim:* The study is aimed at evaluating the chemosensitization and apoptotic effect of aglycone rich extracts of dietary phytoestrogens (derived from soybean and flaxseed) on estrogen receptor positive, MCF-7 and estrogen receptor negative, MDA-MB-231 cells. The extracts show potent activity on both the cell lines, hence, in silico studies have been carried out to find the possible reason for their activity.
*Main methods:* MTT assay was carried to assess chemosensitization effect and activated caspase-3/7 activity was studied using flow-cytometry and western blotting. In silico studies were carried out using PharmMapper and the top hits were taken up for docking using the Schrödinger software. Top molecular targets were subjected to gene expression studies by qPCR and protein expression using Western blot analysis.
*Key findings:* This study reports the apoptotic activity and chemosensitization effect of the phytoestrogens. Molecular docking studies predict AKR1B1 (aldose reductase), HRAS (Harvey rat sarcoma) and GSTP1 (glutathione s-transferase pi) as potential molecular targets for genistein, daidzein and secoisolariciresinol, respectively. Gene and protein expression studies show down-regulation of AKR1BI, HRAS and GSTP1 by the extracts.
*Significance:* The qPCR and western blot analysis results support the computational analyses, and hence genistein, daidzein and secoisolariciresinol may be considered as good candidates for future development into potent inhibitors of the respective protein targets through medicinal chemistry optimization.


## 1. Introduction

Breast cancer is the most common cancer among women in India and the second most common cancer in the world. It is the leading cause of cancer deaths in women [1]. Clinically, breast tumors can be classified on the basis of hormone receptor status, with estrogen receptor-positive (ER+) cancers occurring thrice more often than ER-negative (ER−) cancers [2]. Categorizing breast tumors based on the ER, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status is necessary for predicting the outcome and assist in the management of breast cancer [3]. Triple-negative breast cancers (ER−, PR−, HER2−) show a partial response to chemotherapy and with the present lack of clinically established targeted therapies, have a poor prognosis [4,5]. The systemic agents are active at the beginning of therapy, however over a period of time as progression occurs, the resistance to therapy develops. Besides, these therapies show side effects such as mild dizziness, nausea, loss of fertility and incidence of secondary cancer [6–8]. In order to overcome the problems such as non-selective cytotoxicity and chemoresistance associated with conventional treatment strategies as well as the inherent pro-survival mechanisms of cancer cells, the development of alternative treatment strategies or supplementation to existing treatment is necessary. One such therapy is chemosensitization of cancer cells by plant polyphenols.

Plant polyphenols, especially phytoestrogens, are the focus of our study. Phytoestrogens are xenoestrogens of plant origin which exhibit structural similarity to the mammalian steroid hormone, 17β-estradiol (**1**, Chart 1). They can be classified as i) flavones (e.g., kaempferol (**2**) and quercetin (**3**)), ii) isoflavones (e.g., genistein (**4**), daidzein (**5**), equol (**6**)), iii) lignans (e.g., secoisolariciresinol (**7**), enterolactone (**8**), enterodiol (**9**)), iv) coumestans (e.g., coumestrol (**10**)), v) mycotoxins (e.g., zearalenol (**11**)) and stilbenes (e.g., resveratrol (**12**)) (Chart 1).

The mechanism involved in the phytoestrogen bioactivity may be due to their possible binding to the ERs because of their structural similarity to **1** as depicted in Fig. 1 [9–12]. The binding affinity to ERs is

*Abbreviations:* ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; ROS, reactive oxygen species; SARE, soybean aglycone rich extract; FSARE, flax seed aglycone rich extract; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ATP-TC, adenosine triphosphate-tumor chemosensitivity; $DOX_{IC50}$, $IC_{50}$ concentration of doxorubicin; GTP, guanosine triphosphate; GDP, guanosine diphosphate; PDTD, potential drug target database.

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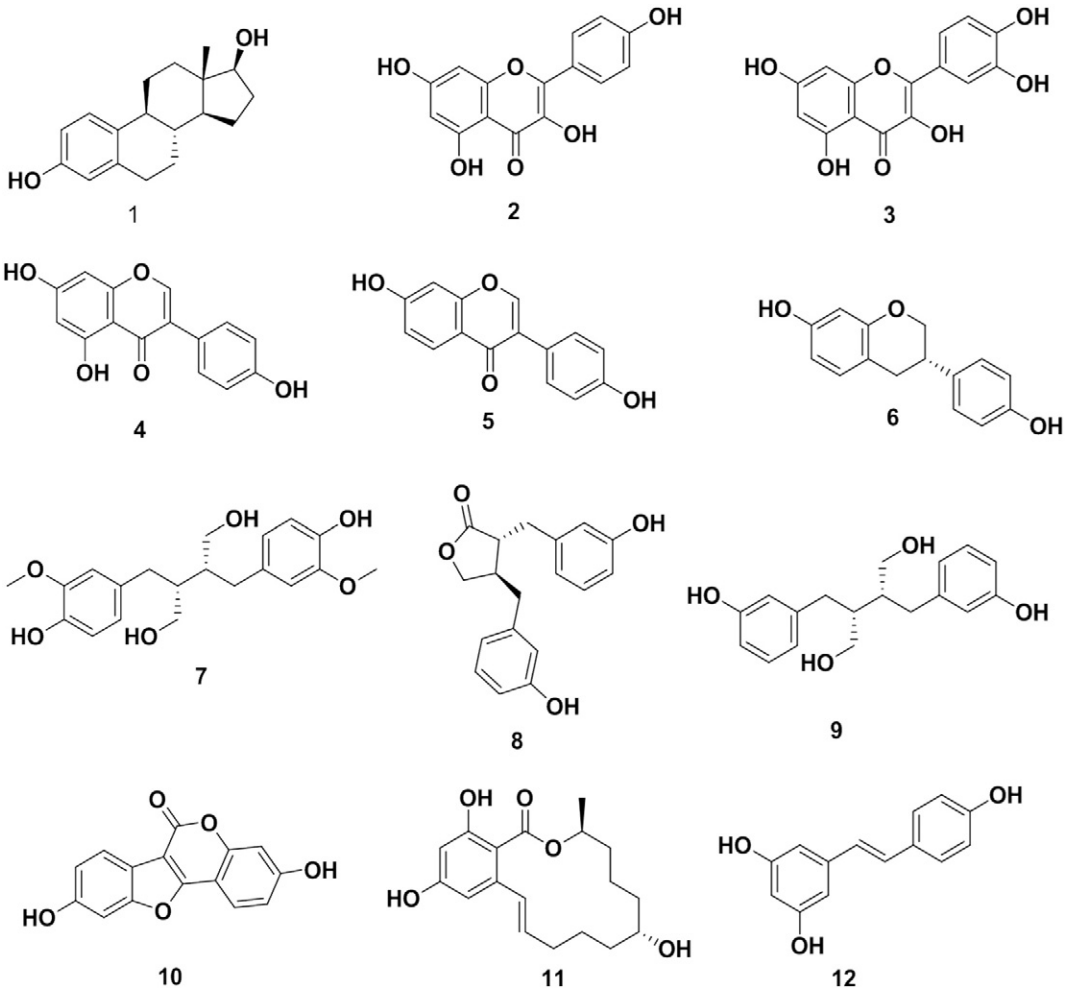


**Chart 1.** Molecular structures of 17β-estradiol and various phytoestrogens.

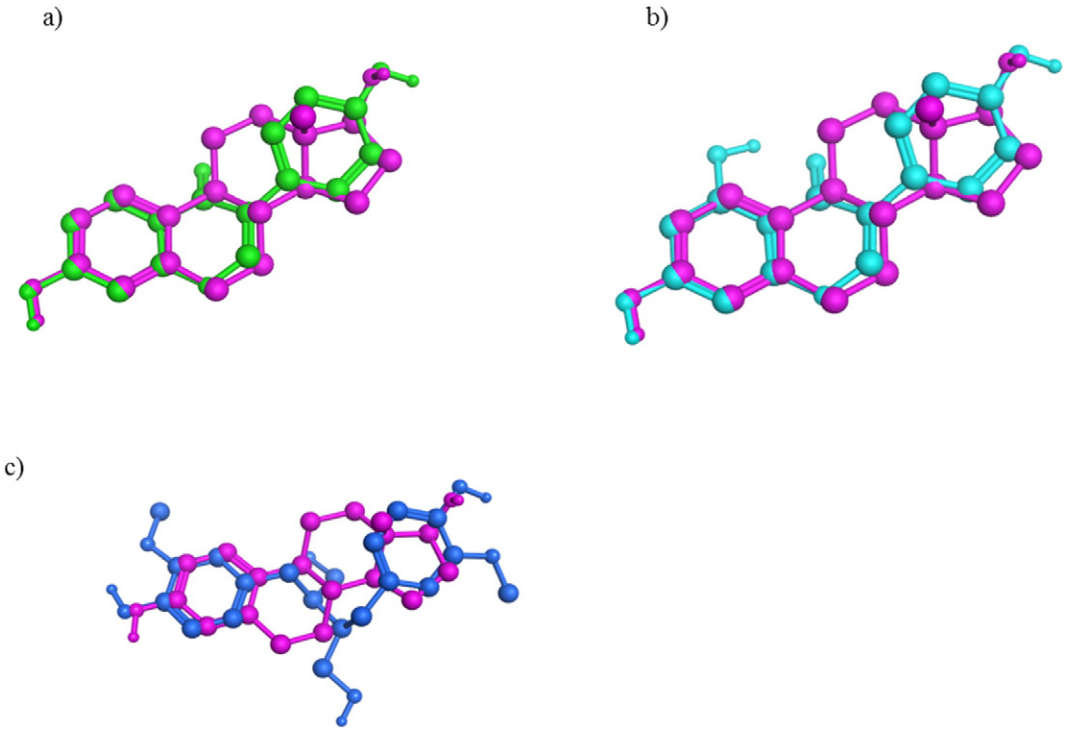


**Fig. 1.** Flexible alignment of a) genistein (**4**) onto 17β-estradiol (**1**) b) daidzein (**5**) onto **1** and c) secoisolariciresinol (**7**) onto **1**.