



A Review on the Role of Phytoconstituents in Breast Cancer Cells

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Abstract: This review deals with the phytoconstituents that could be useful in breast cancer cells and in researches dealing with the development of drug molecules for the management of cancer. It also throws bright to light on herbs like *Aesculus indica*, *Garcinia Mangostana*, *Pfaffia paniculata*, *Sapium ellipticum*, *Solanum nigrum*, *Artemisia vulgaris*, *Vernonia amygdalina*, *Phyllanthus watsonii* and herbal molecules such as vinblastine, vincristine, taxol, elliptinium, camptothecin, irinotecan, topotecan, silvestrol, curcumin, ovatodiolide, limonoids, flavanoids and carotenoids on which research were already carried out successfully against various breast cancer cells. Present review will contribute in promoting more researches leading towards the development of herbal molecules that could be highly beneficial not only in combating this deadly disease but also in providing an eco-friendly safe and efficacious herbal way.

Key words: Breast cancer, medicinal plants, phytoconstituents, MCF7.

1. INTRODUCTION

Cancer is one of the major killing diseases, worldwide and more than 6 million die of the disease and over 22 million people in the world are cancer patients¹. It is predicted that cancer incidences increasing every year in both developed and developing countries². The disease affects men and women alike. Thus, public and private sector institutions are focusing their research towards the development of anticancer agents. Cancer chemotherapy now plays a significant role in treating malignancies, it acts as either curative (by itself or as an adjuvant to surgery and/or radiation) or palliative care, depending upon the specific tumour situation³.

Reports state, there is over ten million new cases of cancer (all sites excluding non-melanoma skin), with over six million deaths, worldwide. Since 1990 there has been a 22% increase in cancer incidence and mortality. Of these cancer cases frequent incidents are lung, breast, colorectal, and stomach cancers. It has been observed that breast cancer is of frequent occurrence and common among women between age group of 45–55⁴. Studies on breast cancer have consistently found an increased cancer risk associated with elevated levels of endogenous and exogenous estrogens in the body⁵. Differences in breast cancer mortality can be seen among worldwide countries and is mainly attributed to the role of lifestyle, especially nutrition. Recent studies suggest that phytoestrogens which are present in fruits, vegetables and whole grain stimulate estrogen tumour⁶⁻⁸.

1.1 Breast cancer cell lines: Their origins and properties

Breast cancer cell lines which have been used in medicinal plant research are characterized into three groups based on their phenotypes and invasiveness⁹. The first group is luminal epithelial-like cells. This group expresses high amount of typical luminal epithelial phenotype of breast cells such as estrogen receptor (ER), E-cadherin (gene CDH1), zonula occludens-1 (ZO-1), desmoplakin I/II (DSP), and desmosomal junctions. This group of breast cancer cells will grow as interconnected colonies of polygonal cells on plastic and as fused colonies in Matrigel, which is semisolid medium. This kind of cells are weakly invasive. BT-483, MCF7, T47D, and ZR-75 are all in this group. The second group is called weakly luminal epithelial-like cells. This group of cells shows the expression closely to the first group with a reduced extent or at least some of those markers. Cells are weakly invasive *in vitro*. In Matrigel, most of these cells grow as non-fused spheres. Moreover, on plastic, they will accumulate in clusters of loosely attached cells and rarely reach full confluency. Breast cancer cell lines in this group are BT-474, CAMA-1, MDA-MB134, MDA-MB361, MDA-MB453, MDA-MB468, and SKBR3. The third group is mesenchymal-like or stromal-like cells. It does not express the markers found in the first and second group. In contrast, it exhibits a high level of vimentin (gene *vim*) which is the marker of mesenchymal cells. These cells have fibroblastoid phenotype on plastic and grow as colonies with large stellate projections in Matrigel. They are highly invasive *in vitro*. MDA-MB435S, MDA-MB231, Hs578T, and BT-549 are classified in this group.

1.2 MCF7 breast cancer cell line

MCF7 is one of the commonly used breast carcinoma cell line. It is a relatively resistant to cisplatin treatment¹⁰. Morphology of this cell line exhibits epithelial-like cell including the ability to process estradiol via cytoplasmic estrogen receptors and dome formation. This cell line has oncogene, *wnt7h* and can induce tumor in nude mice. MCF7 cell line expresses both estrogen and progesterone receptors whereas the expression of Her2/neu is absent⁹. The cells can be suppressed by catechin hydrate, product from plant sources such as green tea, through TP53/caspase mediated apoptosis¹¹. Some vasoactive peptides such as endothelin 1 is found at low level in MCF7 while in SKBR3 it is expressed at the higher level. This expression might correlate with high invasiveness phenotype in breast cancer¹² [Kulbe et al.,]. MCF7 cells have been extensively used as the model for breast cancer and breast cancer therapy. However, different sources of MCF7 show difference in response to 17beta-estradiol, resulting from activation or inhibition of insulin-like growth factor I (IGF-1)¹³. Therefore, the different responses of MCF7 should be realized due to the expression of IGF-1. Hence it is delineated that, MCF7 is suitable candidate as reference materials in quality control for HER2 testing¹⁴.

1.3 Herbs in the management of Breast Cancer

Since time immemorial plants have been used as a source of medicine for treating various ailments¹⁵. World Health Organization estimates that majority of the people depend on herbals for their health care. As for cancer protection, it has been studied that diets rich in phytochemicals can reduce the risk of cancer by 20%¹⁶. Plant natural product chemistry has played an active role in generating a

significant number of drug candidate compounds in drug discovery program. It has been reported approximately 49 % of 877 small molecules were introduced as new pharmaceuticals between 1981 and 2002 by New Chemicals Entities either as natural products or semi-synthetic analogs or synthetic products based on natural product models. Secondary metabolites and their derivatives from plants and other natural resources have been used for the treatment of various diseases since thousands of years¹⁷.

Medicinal plants can be a promising source of novel chemotherapeutic agents especially for cancer. It has been estimated that, out of total 250,000 plant species existing on earth approximately one thousand species are known to have anticancer potential. Thousands of plant species have been screened through bioassays for search of novel plant based anticancer drugs¹⁸. Bioactivity guided isolation is an important strategy for discovery of potent anticancer agents¹⁹.

Aesculus indica (Linn.) is a large deciduous tree distributed in northern western Himalayas. The seeds, fruits and roots of the plant are traditionally used against many ailments. Leaf extract of the plant found to possess antioxidant property²⁰, stimulate cell mediated immune response and apoptosis induction in breast cancer cells²¹.

The studies revealed that *Garcinia mangostana* (GM) has anti-inflammatory²², antitumour, antioxidant and antibacterial activities²³. GM has long been served as traditional medicine, very few authentic scientific studies are there for anticancer property of this plant. A study has revealed the presence of xanthone, mangostin and tannin in the pericarp of GM. Tnanin, in particular, present in the plant, was found to be an inducer for apoptosis on human breast cancer cell line²⁴.

Pfaffia paniculata (Brazilianginsengor Suma) is a native shrubby medicinal plant of Brazil and have been well documented for various therapeutic values. This plant has also been used for cancer therapy in folk medicine. Data clearly indicated that butanolic extract of *P.paniculata* is cytotoxic, exhibiting mitochondrial damage and damage cell membranes and nuclear structure. This study warrants further in-depth investigations on to prove this plant source as a potential source of chemopreventive/chemotherapeutic agent²⁵.

Water extract of *Solanum nigrum* and *Artemisia vulgaris* revealed inhibitory effect against breast cancer cells. Similarly, extracts of *Cichorium intybus*, *Smilax glabra*. *Solanum nigrum* and *Swertia chirayta* showed a modest inhibition against MCF 7, RKO and PC-3 cell lines. This cancer cell line inhibiting property may be attributed to either apoptosis induction or DNA fragmentation or caspase -3 activation or poly (ADP) ribose polymerase cleavage caused by these extract²⁶.

Alkaloid and terpenoid fractions present in the stems of *Berberis aristata* DC and *Hemidesmus indicus* R.Br rhizomes, revealed potent cytotoxic activity against MCF-7 breast cancer cell line²⁷

Traditional medical practitioners, herbalists, and local healers in West Africa recommend aqueous *Vernonia amygdalina* (VA) for their patients to treat various diseases. Although studies indicate that VA is effective against MCF 7 cells. VA leaf extracts slightly ($P < 0.05$) reduce cellular viability and induce minimal DNA damage in MCF-7 cells as assessed by MTT and alkaline single cell gel electrophoresis assays, respectively. Findings from *in vitro* studies provide the pharmacological support that VA represents a potential DNA-damaging anticancer agent against breast cancer²⁸

Soybean being a rich source of dietary isoflavones has been investigated extensively with mutually contradictory indications. Fenugreek is a widely used spice with a long history of medical uses in Ayurveda and Chinese medicine and reported to have high flavonoid content with antioxidant and chemopreventive properties. Results show that, fenugreek extract induces apoptosis in MCF-7 cells²⁹.

Dioscorea membranacea Pierre, *Dioscorea birmanica* Burkill (Dioscoreaceae) and *Siphonodon celastrineus* Griff. (Celastraceae) plants were studied for their anticancer efficacy. The extracts were tested against breast cancer cell line MCF 7, human large cell lung carcinoma (COR-L23), human colon

Adenocarcinoma (LS-174T), and one normal human keratinocyte cell line (SVK-14). The results indicated that these three plants exhibited high cytotoxic activity against these cell lines³⁰.

Recent studies reported that consumption of fruits and vegetables may not have a significant influence in reducing the risk for breast cancer³¹. However, several bioactive compounds derived from fruits and vegetables, including flavonoids³²⁻³³ polyphenols³⁴ and vitamins were evaluated for inhibition of breast cancer cell growth and metastasis in *in vitro* and *in vivo* model systems.

Citrus fruits, one of the major contributors of human diet, received researcher's attention recently due to their bioactive compounds. Bioactive compounds from citrus, such as limonoids, flavonoids (naringin), and carotenoids (lycopene, lutein), were effective in suppressing the growth rate of human breast cancer³⁵ colon cancer³⁶ neuroblastoma cells³⁷ and rat prostate carcinoma cell. It is well known that cancer occurs due to either mitochondria-generated reactive oxygen species (ROS), DNA damage, apoptosis, or necrosis³⁸.

Solanum nigrum L. (SNL) has been traditionally used in Oriental medicines and is believed to have various biological activities. For example, in the Indian traditional medicine, the plant is used as hepatoprotective agent, and the fruit of SNL is also used as a nervous tonic in the Mexican medicine³⁹. A study reported that SNL ethanol extract suppressed the oxidant-mediated degradation of calf thymus DNA⁴⁰. The studies suggest that SNL possesses a beneficial activity as anti-oxidant and anti-tumour promoting agent, although the mechanism for the activity remains to be elucidated.

Phyllanthus watsonii (*P. watsonii*), a small shrub growing to about 1 m high, is one of the three plants in the genus that is endemic to Peninsular Malaysia²⁸. The S phase arrest in MCF-7 induced by *P. watsonii* at a concentration of 10 µg/ml may be related to the down regulation of *cyclin A* and *cdk2*. The *in vitro* cytotoxic activity of *P. watsonii* extract against MCF-7 human breast cancer cells, were highly positive. The results demonstrated that *P. watsonii* has strong cytotoxic effects by inducing apoptotic cell death, increasing caspase-3 activity, and causing S phase arrest in cancer cells⁴¹.

Species of the genus *Phyllanthus* are common non toxic plants found in tropical countries with high therapeutic values including anti-HIV and anticancer potentials⁴².

Ovatodiolide, a cembrane-type diterpenoid, is the major component of *Anisomeles indica* (L) has been reported to exhibit cytotoxicity against human cancer cell lines⁴³ and is dependent on both intrinsic and extrinsic pathways⁴⁴.

1.5 Plant molecules as anticancer agents

Few molecules isolated and identified from traditional drug sources and proven as anticancer drug will be discussed in sequel.

The isolation of the vinca alkaloids, vinblastine(1) and vincristine(2) from *Catharanthus roseus* G. Don were used along with other cancer chemotherapeutic drug for treating a variety of cancers. The discovery of paclitaxel (Taxol, 3) from a traditional drug source, is another evidence of the natural product drug discovery for cancer. *Taxus baccata* was reported to be used in the Indian Ayurvedic medicine for the treatment of cancer. Paclitaxel, a bioactive molecule isolated from this plant drug is significantly active against ovarian and breast cancer⁴⁵.

Elliptinium (4), a derivative of ellipticine, isolated from a Fijian medicinal plant *Bleekeria vitensis* A.C. Sm., is useful in France to treat breast cancer⁴⁶ [Cragg and Newman, 2005]. Camptothecin (5), an anticancer drug from *Camptotheca acuminata* Decne (Nyssaceae), was dropped after clinical trial due to severe bladder toxicity¹⁷ [Potmesil et al., 1995]. Semi-synthetic derivatives of camptothecin like Topotecan (7) and irinotecan (6) were also useful as cancer drug molecules. Silvestrol (8) is yet another phyto molecule useful against breast cancer⁴⁷.

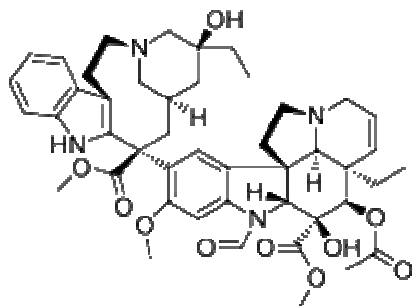
Most investigations on anticancer agents from medicinal plants were based on their ethnomedical use and their active molecule identified and synthesized lead to the development of potent anti-cancer drugs. Researchers have investigated the use of whole plant extract and subjected them to evaluate their anticancer potentials and they also attempted to identify active molecules.

In contrast, using herbal preparations as such has produced gentler effects on the human body because of the synergistic effect of the chemical constituents present in the preparations. It is conceivable that anticancer drugs in future will consist of an anticancer herbal formula composed of many medicinal plants. Each plant will contribute to the management of the disease in various ways such as enhancing the immune response or providing pharmacological effects. Therefore, it is likely that in the predictable future there will be a major breakthrough in the development of anticancer drugs from traditional drug sources.

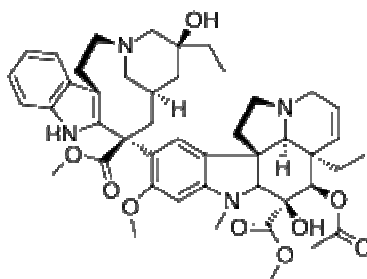
FUTURE SCOPE

Latest statistical analysis on breast cancer predicts that cancer incidences are increasing every year in both developed and developing countries causing serious health hazards particularly to women. Such kind of studies carried out on literature survey will certainly contribute in identifying newer herbs and novel molecules which could be safe and efficacious in the management of breast cancer.

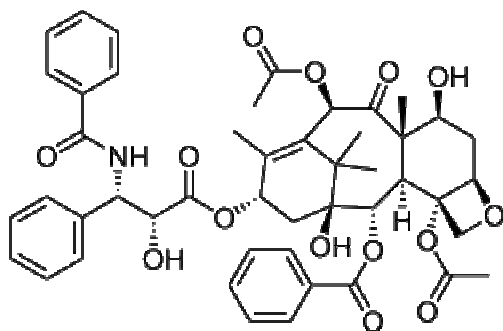
Few Important anticancer drug molecules from traditional plant sources



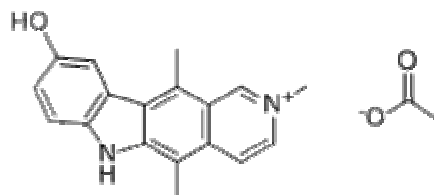
1. Vinblastine



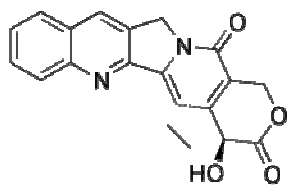
2. Vincristine



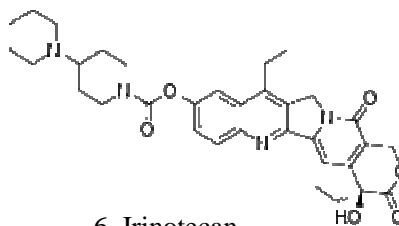
3. Taxol



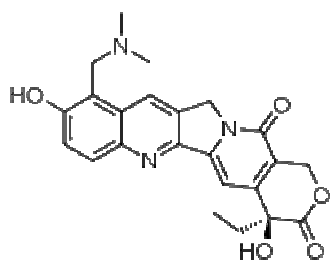
4. Elliptinium



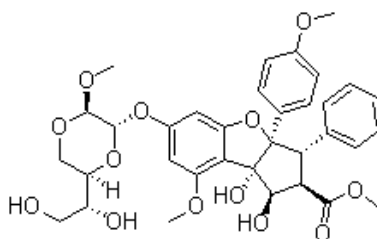
5. camptothecin



6. Irinotecan



7. Topotecan



8. Silvestrol

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