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Cucurbitacin Compounds Against Estrogen Receptor: Literature Review

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ABSTRACT

Over the past few decades, extensive research in the field of carcinogenesis has been the toughest challenge in finding newer drugs. One of the leading causes of death in women worldwide is breast cancer. Cucurbitacin is one such compound identified to suppress the oncogenic signalling pathways for survival. JAK/STAT pathways were identified for tumour growth as one of the key targets for cucurbitacin. Mainly, the compound cucurbitacin Q against estrogen receptors could be a target of concern among researchers around the globe. The structured review of cucurbitacin was documented by retrieving the data from various literature reports, review articles and research papers published on the PMC platform. In context with the fascinating role of cucurbitacin Q against estrogen receptors, it inhibits the tumour progression by blocking the STAT3 pathway. Cucurbitacin Q induces apoptosis in the tumour that activates the STAT3 gene when compared to other genes, which were found to be susceptible to breast cancer cell lines. Therefore, Cuc Q finds itself a new way of intervening with the JAK/STAT3 pathway by suppressing the progression of the tumour. Increased production of Cuc Q if proved to be active against oncogenes by blocking the STAT3 pathway. This article discusses the background, chemical structure and biological mechanism of cucurbitacin Q compound against estrogen receptors for breast cancer treatment.

KEY WORDS: CARCINOGENESIS, CUCURBITACIN, ESTROGEN RECEPTOR, MECHANISM AND ONCOGENE.

INTRODUCTION

Medicinal plants are found all over the globe for the benefit of mankind. The plant consists of various secondary metabolites found with different compositions in various parts of the plant. Mainly potential secondary metabolites from the traditional medicinal plant are responsible for different disorders, ailments and other treatments. Natural products are familiar for exerting anti-tumour activities partly based on their ability to lessen ROS (Reactive Oxygen Species) and to defend critical cellular components like DNA, proteins and lipids from oxidative damage (Rafter 2002). The pharmaceutical market needs to be updated with newer drugs for developing effective treatments against deadly diseases (Gupta and Kohli 2019). Developing treatment plans for cancer is an unending struggle, but relapses and treatment-related complications continue as the main impediment (Miladiyah et al. 2020).

To eliminate the possible failures in the drug development stage several approaches such as *in silico*, *in vitro, in vivo*, and cell lines are being practised (Umar et al. 2020). Estrogen receptor alpha (ERα) and progesterone receptor (PR) are found in cancer cells of the breast. If the breast cancer cells have estrogen receptors, the cancer is called ER-positive breast cancer (Altwegg and Vadlamudi 2021). ER, signalling is a key driver of ER + breast carcinogenesis and inhibition of ER signalling is the mainstay of ER + BC therapy and has enhanced the patient's survival rate (Scabia et al. 2022). This review focuses on E2 binding to membrane-bound ERα and ERβ receptors that swiftly stimulate nuclear transcription factors via the MAPK pathway and other pathways involved. The rationale of the study includes molecular targets, especially like JAK2/STAT3 pathway for tumorigenesis where such cucurbitacin compounds could prove to inhibit these pathways (Scabia et al. 2022).

Cancer: Cancer is a disease resulting in abnormal growth of cells and uncontrolled multiplication of cells within the body (Pushpalatha et al. 2017). According to World Health Organization (WHO), cancer is one of the second main reasons of mortality around the globe with about 9.6 million death in 2018 (Bray et al. 2018). Mostly the cancers are treated through chemotherapy. Drugs from herbal sources are low in toxicity, low cost and bioavailable. The most common cancers are lung, liver, colorectal, stomach and breast cancer (WHO 2017; Scabia et al. 2022).

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The International Agency for Research on Cancer (IARC), a unit of WHO reported that 28 types of cancer are found in 184 countries and this is alarmingly increasing (Kumar et al. 2020). Breast cancer (BC) is the most common sort of tumour mostly in females, yet metastases are the key reason for deaths (Cava and Castiglioni 2020). Breast cancer chemotherapy is marked by pointing to the role of receptors such as ERα (Estrogen Receptor alpha), PR (Progesterone Receptor), EGFR (Epidermal Growth Factor Receptor) (Acharya et al. 2019). Sahayarayan et al. (2021) reported that over 60% of breast cancer cases are diagnosed as estrogen receptor alpha positive (ERα) cancers mainly in Asian countries. In humans, both alpha and beta estrogen receptors are revealed and many studies are focused on these ER receptors (McDonnell et al. 2015).

Regarding the issue, many researchers have focused on finding highly sensitive and specific markers for the initial detection of breast cancer (Yan et al. 2015). Moreover, the anticancer effects of cucurbitacin compound on different tumour types like neuroblastoma, breast cancer, lung cancer, endometrial cancer and hepatocellular carcinoma have been well studied and documented (Si et al. 2019). We provide a framework of the main estrogen receptor and various oncogenic pathways which regulates the processes of human tumorigenesis (Scabia et al. 2022).

Estrogen Receptor (ER): In humans, alpha and beta estrogen receptors were described, and many studies focused on these receptors (McDonnell et al. 2015). The increased production of estrogen is one of the main foremost causes of breast cancer (Sahayarayan et al. 2021). In women, ER (Figure 1) plays a vital part in apoptosis, inflammation, homeostasis, differentiation, maturation, metabolism and proliferation in breast cancer (Bai and Gust 2009). Several studies have reported that estrogen, in specific 17 β-estradiol, has been reported to up-regulate the expression. Also, the purpose of c-Myc and cyclin D1 genes is to lead the promotion of the cell cycle from G1 phase to S phase in the epithelial cells of mammary glands (Acharya et al. 2019; Scabia et al. 2022).

Figure 1: Three-dimensional (3D) structure of Estrogen receptor protein (PDB ID: 3ERT)

The hyperactivity of ER-α in the mammalian cells leads to the conservation and growth of types of breast cancers and also holds many molecular targets for the study of cancer drugs (Sahayarayan et al. 2021). Peng et al. (2009) reported that nearly 60% of pre-menopausal women and about 75% of post-menopausal women have suffered from estrogen-dependent breast cancer, and ER-α activity was efficiently inhibited using cancer therapy. Even though a lot of anticancer drugs and potential inhibitors against various targets are available, the effective surge in resistance along with side effects indicates that there is an urgent need for novel tumour therapy (Sahayarayan et al. 2021).

Women with breast cancer who took tamoxifen treatment are at higher risk. They also have an increased occurrence of endometrial cancer but a reduced amount of certain bone fractures and a dramatic 45% diminution in the incidence of breast cancer (Shaiau et al. 1998). Still, a lot more research is to be done, and is tremendous progress toward finding a cure for ER-α of breast cancer. Bernards (2012) reported that the vital issue is the lesser dependability on cell lines that predicts the efficacy of drugs since cell lines didn't confirm to be a perfect model. Therefore, computational methods are in demand to expand the potential role of a drug in the context of the pathway (Cava et al. 2018; Scabia et al. 2022).

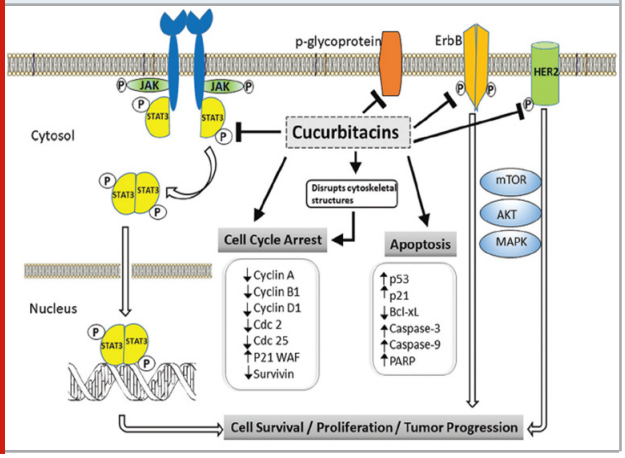
Molecular Mechanism of Cucurbitacin and Cucurbitacin Q: There are many oncogenic signaling pathways that are frequently included in cancer cell proliferation and survival. Recent studies have uncovered that several molecular targets of cucurbitacin such as the JAK2/STAT3 pathway, cofilin, cyclins, cdc2, COX-2, TYR and EcR among which actin cytoskeleton appears to be a prime target (Blaskovich et al. 2003; Chen et al. 2012). The JAK/STAT (Signal Transducers and Activators of Transcription) pathway (Figure 2), Akt-PKB pathway and MAPK Pathway are significant pathways in cancer cells and are also targets of the *Cucurbitaceae* family (Lee et al. 2010). In many cancer cells, activation of STAT3 and STAT5 has been known to play key roles in tumorigenesis (Yu and Jove 2004). During the initial findings, reports revealed that Cuc I is a dual inhibitor of STAT3 and JAK2 pathways but didn't affect any other oncogenic signaling pathways such as Akt-PKB or MAPK (Blaskovich et al. 2003; Scabia et al. 2022).

In cancer cells, cucurbitacin compounds labour as STAT3 inhibitors and make cells prone to the attack of reactive oxygen species (ROS) and free radicals during inflammation (Jayaprakasam et al. 2003). Inhibition of the IKK/NF-kB pathway by cucurbitacin relies on the inhibition of key inflammatory enzymes, like cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) whose overproduction leads to tumorigenesis (Jayaprakasam et al. 2003; Park et al. 2004; Escandell et al. 2007). Although other mechanisms like activation of the MAPK pathway leading to cancer cell proliferation and survival, another study revealed that only STAT3, but not the MAPK pathway which was impaired in breast cancer cells when treated with cucurbitacin E compound (Lan et al. 2013; Alsayari et al. 2018).

However, cucurbitacin F, O, P, and Q and their derivatives were identified to have the finest anticancer activity (Chen et al. 2005). Alghasham (2013) reported that cucurbitacin Q induces apoptosis more effectively in human and murine tumours. It selectively

blocks the activation of STAT3 and induces apoptosis without inhibiting JAK2, Src, Akt, Erk or JNK. A study showed that in nude mouse tumour xenograft model, Cuc Q, but not Cuc A suppresses tumour growth signifying that JAK2 blockage alone is not enough but suggests the competence of Cuc Q to impede tumour growth that is linked to antiSTAT3 activity (Sun et al. 2005). Among the two, Cuc A was shown to be an inhibitor of the JAK2 pathway, whereas Cuc Q induces apoptosis and inhibits tumour growth that contains activated STAT3 (Sun et al. 2005; Scabia et al. 2022).

Figure 2: The mechanistic inhibitory activity of cucurbitacin in pathway (Alsayari et al. 2018)



Bernard et al. (2010) reported that conversion of the C3 carbonyl of the cucurbitacin to a hydroxyl result in loss of anti-JAK2 activity, whereas the addition of a hydroxyl group to C11 of cucurbitacin results in loss of anti-STAT3 activity. Also, Cuc Q persuades cell death more potently in human and murine tumours which constitutively activates STAT3 (A549, MDA-MB-435 and v-Src/NIH3T3) when compared to other (H-Ras/NIH 3T3, MDA-MB-453 and NIH 3T3 cells) (Bernard et al. 2010). Therefore, suppression of oncogene STAT3 seems to be related to blocking the tumour which doesn't eliminate alternate mechanisms (Zhang et al. 2004; Chan et al. 2010b; Yasuda et al. 2010; Scabia et al. 2022).

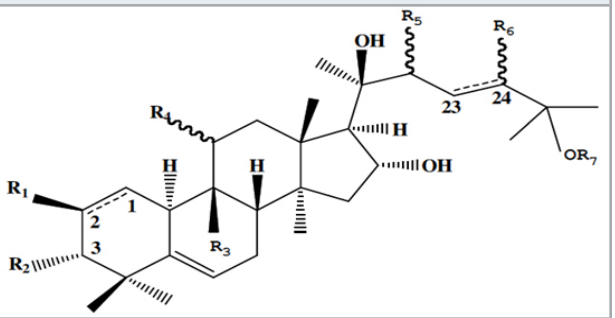
Cucurbitacin Q: Cucurbitacin (Figure 3) are one synthesized chemically by tetracyclic cucurbitane (triterpene hydrocarbon) nucleus skeleton 19-(10à9β)-abeo-5α-lanostane base, altered by the positional replacement of oxygen atom (Sharpless et al. 2002; Kaushik et al. 2015). Overall, there are notably 40 identified species of cucurbitacin and their derivatives, that are classified into 12 groups namely A, B, C, D, E, I, H, Q, R and dihydrocucurbitacin B (Alghasham 2013). Other variations of cucurbitacin are now under exploration for their potential as anticancer drugs (Cai et al. 2015; Garg et al. 2017).

Different studies have revealed by examining the effects of these compounds in several cell lines including *in vitro* and *in vivo* against diverse malignant subtypes. Figure 4 shows the compound cucurbitacin Q (formula: C32H48O8, molecular weight: 560.7 g/mol) identified in the plants of *Cucurbitaceae* and other families with peculiar biological properties (Garg et al. 2017).

Jayaprakasam and co-researchers revealed the cytotoxic properties of cucurbitacin B, D, E and I identified from the fruits of *Cucurbita andreana* against colon, breast, lung and CNS cancer cell lines (Jayaprakasam et al. 2003). Interests in cucurbitacin have developed in recent years and countless studies have demonstrated that analogues of Cus have a wide variety of therapeutic activity that includes, hepatoprotective, anti-cancer and anti-inflammatory activities (Rios et al. 2012). Yet, thorough molecular mechanisms underlying their biological activity remain indescribable (Zhong et al. 2019). A recent study confirmed that *Cissampelos pareira* contains a substantial amount of Cuc Q compound with antiproliferative activity (Amresh et al. 2007; Thavamain et al. 2014). Ali et al. (2019) reported that it doesn't signify that the anticancer potential of the *C. pareira* plant is only due to the existence of a large amount of cucurbitacin Q compound as it requires further research (Ali et al. 2019).

Cucurbitacin Q – Sources: In general, other families of *Scrophulariaceae, Begoniaceae, Primulaceae, Liliaceae, Tropaeolaceae* and *Rosaceae* contain cucurbitacin apart from the *Cucurbitaceae* family (Ali et al. 2019). The seeds of certain cruciferous plants such as Iberis species and *Lepidium sativum* to comprise the cucurbitacin compound (Teuscher and Lindequist 1994; Ali et al. 2019). *Cucurbitacin* Q has been isolated from plants of different families and genera around the globe for research findings (Table 1). The bioactivity of cucurbitacin Q showed its activity on cancer cells in lung A549 human and murine cancer A549, MDA-MB-435 and v-SRV/NIH 3T3 isolated from *Cayaponia tayuya* (Hernandez et al. 2015). Sun et al. (2005) reported that cucurbitacin Q is susceptible to breast cancer cell lines: MDA-MB-435, MDA-MB-453 (Sun et al. 2005; Ali et al. 2019).

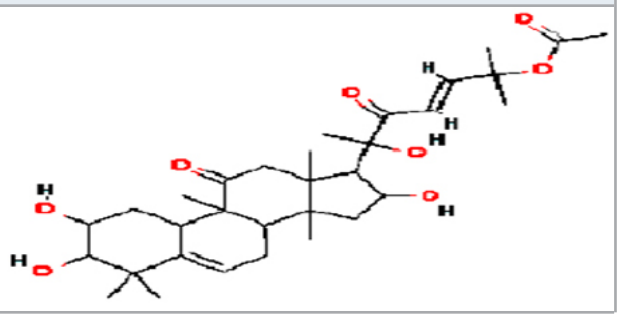
Figure 3: The chemical structure skeleton of main cucurbitacin (Cai et al. 2015)



Cucurbitacin Q – Toxicity: Cucurbitacin belongs to the terpenoids class of various compounds found in the plants of the *Cucurbitaceae* family which has both medicinal and toxic properties (Ali et al. 2019). Gry et al. (2006) reported that the main reason for cucurbitacin being cytotoxic *in vitro* might be due to the compounds influencing cell adhesion to culture vessels. In addition, giving rise to the cytotoxicity of the compound and receptor interaction, isolated cucurbitacin or extracts comprising cucurbitacin have proved to contain biological effects *in vitro* (Gry et al. 2006; Ali et al. 2019). Especially, very minute concentrations of Cuc E and Cuc B, less than 1 µM was shown to inhibit the

adhesion of transformed B cells (Musza et al. 1994; Ali et al. 2019). As Raikhlin-Eisenkraft and Bentur (2000) point that various factors can affect the toxicity of the cucurbitacin compounds. Most of the cucurbitacin-containing plants have been documented where the plants or extracts were screened for cytotoxicity in a battery of human tumour cell lines (Gry et al. 2006; Ali et al. 2019).

Figure 4: Structure of cucurbitacin Q compound



Cucurbitacin Q – Biological Supply Chain and Future Scope: Due to the extreme bitterness of cucurbitacin, plants comprising these compounds would usually not be consumed. The biological activity of cucurbitacin including its pharmacological effect has been analysed from traditional medicinal plants as an active principle (Gry et al. 2006; Ali et al. 2019). The existence and production of cucurbitacin Q have to be extensively studied in future and also about the efficiency of the drug. Over the last few decades, the Cuc Q compound has been shown to block the STAT3 pathway which inhibits tumour progression. Another prominent modification was that cucurbitacin researchers began to examine the biological mechanism of action of cucurbitacin at the molecular level (Lee et al. 2010; Ali et al. 2019).

***In silico* Analysis of Compounds on Breast Cancer Cell Receptors:** A study on molecular docking of compounds that reported its presence in fungal endophytes of *Chaetomium* sp as one of the cytotoxic agents against breast cancer protein (HER α – 1G50). The results revealed that 2 compounds bearing xanthone and benzonaphtyridinedione scaffolds as hit ligands (Hariono and Rollando 2016).

Tamoxifen is an antagonist of ER-α and commercially available as a medicine to inhibit the growth of breast cancer (Jordan 1992). It binds with ARG 394 and blocks the role of ER (Desai et al. 2012). Recently, screening of bioactive compounds from *Phyllanthus emblica* namely quercetin, kaempferol, kaempferol 3-bita-D-glucopyranoside, isocorilagin and 1,1-diphenyl-2-picrylhydrazyl which showed good binding affinity of -7.57 kJ/mol, -7.66 kJ/mol, -6.77 kJ/mol, -7.90 kJ/mol and -5.06 kJ/mol respectively against ER (3ERT) protein (Afrin et al. 2018). A recent study showed that among the phytochemicals like anthocyanins, isoflavone and carnosol with ER, carnosol compound revealed to inhibit with higher binding affinity of -12.1 kJ/mol (Pandian et al. 2014). Among the compounds, SANDB_11243993 has the highest binding affinity of -14.253 kcal/mol against 3ERT protein (Sahayarayan et al. 2021). Paclitaxel, a compound showed good binding interactions with the target proteins in the order ER > PARP 1 > AKT 2 > CDK 6 > HER 2 (Kumar et al. 2020).

Table 1. Potency of cucurbitacin Q against cancer

S. No	Plant sources	Presenting condition	Activity explored	References
1. 2.	*Helicteres isora* *Anagallis arvensis*	anticancer	Anti-tumour/ STAT3 pathway	Cai et al. 2015
3. 4.	*Gurania Subumbellata* *Picrorhiza Kurrooa*			
5.	*Cissampelos pareira*	anticancer	Anti-tumour	Bala et al. 2015, Bala et al. 2019
6.	*Citrulllus colocynthis*	anticancer	Anti-tumour	Al-Snafi 2016, Hussain et al. 2014
7.	*Wilbrandia species*	anticancer	Anti-tumour	Matos et al. 1991
8.	*Ecballium elaterium*	anticancer	Anti-tumour	Chen et al. 2005

In our current study, molecular docking of protein with ligands were studied to analyze the interactions and among the cucurbitacin compounds, cucurbitacin Q has shown to inhibit the ER-α protein (3ERT) which showed maximum docking score of -9.3 kcal/mol. Among the compounds, cucurbitacin paved a way much into the development of drug in near future. Predicting the drug candidates for pharmacokinetic and dynamic profile early in the drug development preparation which is the key aspect of ADME was tested using SWISS software (Daina et al. 2017). Molecular dynamic simulation studies of the protein-ligand complex are under process using the NAMD software (Phillips et al. 2005) to predict the stability of the molecule or compound (Ali et al. 2019).

Cucurbitacin Q – *In vitro* Production Studies: To increase the biomass and yield of cucurbitacin Q, *in vitro* production studies have to be implemented from traditional medicinal plants using plant tissue culture technology as an alternative source. Enhanced accumulation of total cucurbitacin content was shown to be higher than the parent plant. This *in vitro* secondary metabolite production from medicinal plants was considered a suitable alternative method compared to whole plant extraction (Devendra

et al. 2012). Till now up to date, there is no report on increased production of cucurbitacin Q compound from medicinal plants. Further, investigation of the effect of plant growth regulators and elicitors plays a vital role in the biosynthesis of cucurbitacin Q and their intermediates (Ali et al. 2019).

Biosynthetic Pathway of Cucurbitacin: Balliano et al. (1983) have explored the biosynthesis of cucurbitacin glycosides from squalene-2,3-epoxide to the final cucurbitacin, aiming at the possible routes for biosynthesis (Figure 5). The occurrence of 10α-cucurbita-5,24-dien-3β-ol in many seeds of food plants has been revealed. This compound is now considered a primary intermediate in the biogenesis of cucurbitacin (Akihisa et al. 1986; Ali et al. 2019). The most probable change of lanostane C-9 carbonium ion (3) to cucurbita-5,24-dienol (5) was examined as the precursor. The fact that two pentacyclic compounds, glutinol and simiarenol are frequently found together with cucurbita-5,24-dienol is taken as support for the biosynthetic route of triterpenoid compounds (Balliano et al. 1983; Ali et al. 2019).

CONCLUSION

The findings of the present reiew has shown that over several decades, this neglected compound is gaining attention as a potential anticancer drug. Cucurbitacin Q is an under-explored compound which can be verified and worth due to its cytotoxic potential activity against cancer and other activities. Through this study, the suppression of STAT3 and JAK2 in the JAK/STAT pathway can be deactivated by inhibiting its process using Cuc Q and Cuc A and is an excellent candidate for clinical investigation. Moreover, studies related to the genetic mice tumour model should be considered to assess the active anticancer activities of cucurbitacin Q in the STAT3 pathway which inhibits tumour progression. Subsequently, it caused growth arrest, apoptosis, cellular differentiation and blockage of proliferation in cancer cells. Finally, clinical trials for Cuc Q as the targeted compound for the anticancer agent as an independent effector.

Conflicts of Interests: Authors declare no conflict of interests to disclose.

Data Availability Statement: The authors declare that the information provided in this paper is available and can be shared when required based on the request made to the corresponding author.

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