Chapter from the book *Phytochemicals - Isolation, Characterisation and Role in Human Health*

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1. Introduction

1.1. Phytochemicals in the cucurbitaceae

The seeds of several species are a rich source of proteins, lipids, unsaturated fatty acids (table 1), phytosterols, vitamin E, and some minerals such as Mn, Zn, Cu, and carotenoids. The flesh of the fruits also contains some important compounds, such as flavonoids, that show antioxidant activity.

Other phytochemicals in the cucurbits are scarce, but polysaccharides is important to mention. These compounds, bound to proteins, are often considered as key active compounds in some species particularly with regards to diabetes. Alkaloids and saponins have been described in *Momordica* and *Sechium*, and also polyamines and galactolipids. In *Sechium*, the presence of proteins with ribosome-inhibiting activities has been described.

<table>
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<th>Water (%)</th>
<th>Calories (kcal)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrate (g)</th>
<th>Fiber (g)</th>
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Table 1. Nutrient composition of Cucurbitas in 100-g edible raw portion

2. Cucurbitacins

The cucurbitacins, as characteristic compounds of many species of the cucurbit family, are tetracyclic triterpenes arising from a rearrangement of the protostane cation. They are unsaturated and polyfunctional oxygenated compounds and occur most often as glycosides. They are particularly toxic substances, the bitterness and cytotoxicity being the contributing factors for this toxicity [1].

They are divided into twelve groups, from cucurbitacin A to cucurbitacin T. The cucurbitacin I, B, D, E, and L the most used in vivo and in vitro studies and differ by the acetylation of groups OH or by the presence of double bond (Figure 1) that increase the lipophilic and toxic properties [2, 3].
Figure 1. The chemical structure of major cucurbitacins

3. Physical properties and solubility of cucurbitacins

Some of the cucurbitacins are crystalline solids, but others are gums or semisolids and their structure contribute to their scarce solubility in water and this factor is a challenge in pursuing their biological activity. There are some studies [4, 5, 6] that tried to search how to deliver the active principle without this problem, such as the use of polymeric micelles for nanoscale drug delivery. The amphiphilic block copolymers has the capacity to accommodate several types of molecules for drug delivery and the poly(ethylene oxide)-block-poly(ε-caprolactone) called PEO-b-PCL, which is a biocompatible copolymer successfully used for the solubilization of compounds of poor solubility in water, is suitable for solubilization and controlled delivery of cucurbitacin I and B [6].
4. Bioactivity of cucurbitacins

Besides their cytotoxic and anticancer activity, they also show other pharmacological effects in vivo as in in vitro, for example, as purgative, anti-inflammatory, cough, flu, fever, or anti-fertility. They also show other functions as plant growth regulators and antifeeding agents in insects. A combination of cucurbitacins B + E glucosides showed a notorious antioxidant activity and it is believed that their beneficial properties are due to their ability to interact with reactive oxygen species [10]. It is known that nitric oxide has various functions, but its uncontrolled production can be toxic in many pathological conditions, such as the inflammatory tissue damaged, and in this connection some members of the Cucurbitaceae family have been tested as potential inhibitory agents for nitric oxide production [11]. From Hemsleya pengxianensis, Dihydrocurbitacin F-25-O-acetate was isolated as one of the main components and it was shown that this compound plays a main role as an anti-infection agent [12].

It was shown that at the molecular level, the cucurbitacins have a role in the inhibition of the JAK/STAT3 pathway that is a major contributor in oncogenesis and five cucurbitacins were tested, A, B, E, I, and Q, and found that the last one inhibit the activation of STAT3 but not JAK2, instead cucurbitacin A inhibit JAK2 but not STAT3 and the cucurbitacins B,E, and I inhibit the activation of both. Furthermore, cucurbitacin Q but not A induces apoptosis and inhibits human tumor growth in mice [13]. To validate these interesting results, another model was described in the Sézay syndrome(Sz), which is an aggressive lymphoma/leukemia of the skin, that used cucurbitacin I to inhibit the JAK/STA3 pathway [16] and it was demonstrated that inhibition of STAT3 using cucurbitacin I induced apoptosis of the cells and it was mentioned that this inhibitor of STAT3 is a potent therapeutic agent for Sz.

Cucurbitacin B and R isolated from Cayaponia tayuya induced dramatic changes in the cytoskeleton, inhibiting proliferation and induced apoptosis of isogenic colon cancer cell lines HCT116 and Hke-3 [14]. The cucurbitacin glucosides extracted from Citrillus colocynthis leaves effects on human breast cancer cell growth and it is suggested that this kind of glucosides exhibit pleitropic effects on cells causing both cell cycle arrest and apoptosis [13].

Also, the cucurbitacin E has demonstrated some interesting results because it has emerged in one empirical screening strategy to define agents with potent growth inhibitory activity. It was demonstrated that one early effect of this cucurbitacin is the rearrangement of the actin and the vimentin cytoskeleton. The growth inhibitory actions of a series of cucurbitacins correlate with these effects and that the actin and vermitcin cytoskeleton are potential targets for this kind of compounds [17].

In another model with cucurbitacin B isolated from Trichosanthes cucumerina, it was demonstrated that this compound has a cytotoxic effect on breast cancer cell lines SKBR-3 and MCF-7 and that growth inhibition was attributed to G2/M phase arrest and apoptosis and also that the cucurbitacin B mediates its effect by inhibiting the translocation of β-catenin and galectin-3 proteins to the nucleus rather than by disrupting the β-catenin/galectin-3/TCF-4 complex formation. This means that cucurbitacin B targeting of the Wnt signaling pathway could be an innovative approach for breast cancer treatment [19].
It was observed that when an antitumor agent, such as doxorubicin (DOX), is combined with some natural products such as capsaicin derivatives, gingerol, ferulic acid, or cucurbitacin E, it has a notorious effect on tumor cells. The first ones did not change DOX permeability in the tumor cells but instead cucurbitacin E significantly promoted DOX influx into the tumor cells [18].

In another model, the induction of cancer cell-specific apoptosis via activation of TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) signaling has become an important focus of cancer research and in this sense it was found that cucurbitacins B and D were among the sensitizers of cancer cells to TRAIL-mediated apoptosis in a high-throughput screen [32]. It was found that sensitization by these cucurbitacins is rapid and persistent, making them potentially useful reagents for developing increased understanding of the sequence of molecular events that can lead to TRAIL sensitization and subsequent apoptosis.

The model of proteasome is another approach to understand the molecular mechanism of chemotherapeutic agents. It was found that the proteasome is an abundant catalytic complex present in both nucleus and cytoplasm of eukaryotic cells. Proteasome-mediated degradation plays an essential role in the regulation of most intracellular proteins such as NF-κB and recently proteasome inhibitors have been used as a new anticancer therapy. In this sense it was found that cucurbitacin D induced apoptosis through suppression of proteasome activity both in vivo and in vitro, making this compound a promising candidate for clinical applications in the treatment of T-cell leukemia [20].

5. Response of cancer cells to cucurbitacin exposure

Like other plant-derived substances, cucurbitacins induce toxicity in different cancer cell lines with several morphological and physiological changes (Table 2). Drastic changes in cell shape, such as rounding, swelling, pinocytic blebbing, submembranous inclusions, and blisters, are observed within a couple of hours. Some of the morphological changes could be explained by the dysregulation of cytoskeleton homeostasis by cucurbitacins. Duncan et al. reported a dramatic increase in F-actin to G-actin ratio and abnormal reorganization of the vimentin network by cucurbitacin E in human prostate cancer cell lines [17, 28]. Studies with cucurbitacin B also showed the aggregation of F-actin in various human cancer cell lines [29, 30, 31], implying the disruption of the dissociation process of F-actin by an unknown mechanism. However, unlike vinca alkaloids and taxanes, there is no clear evidence that cucurbitacin affects the microtubule network.

Multinucleation is another common morphological change that was consistently reported in human cancer cell cultures exposed to cucurbitacin for more than 24 h. According to Duncan et al., multinucleation implies that cucurbitacin blocks cytokinesis, but not karyokinesis [17]. This is in conjunction with the observation that actin (which is involved in cytokinesis) is disrupted, whereas microtubules (which are involved in karyokinesis) are not.

Multinucleation can result from the disruption of the cell cycle. Many reports showed that cucurbitacins induced cell cycle arrest, mostly in the G2/M phase [31, 32, 33, 39], but S-phase
arrest in HL-60 and U937 human leukaemia cell lines was also reported [11]. G2/M arrest happens in the early period of cucurbitacin exposure and results in apoptotic death of the tumor cells [39]. Tannin-Spitz et al. showed that G2/M arrest occurred in breast cancer cell lines (MCF-7 and MDA-MB-231) exposed to cucurbitacin B/E glucosides by the inhibition of cyclin-dependent kinase (cdk) p34\(\text{CDC2}\) and cyclin B1, both in expression level and activation status [33]. G2/M has shown arrest by upregulation of cdk inhibitor p21\(\text{WAF1}\), and by downregulation of cyclin A and cyclin E in pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) exposed to cucurbitacin B [32].

6. Cucurbitacins and their molecular mechanism of action

By what molecular mechanism do cucurbitacins achieve cell cycle arrest, apoptosis, and growth suppression of cancer cells? There are several oncogenic signaling pathways that are commonly involved in cancer cell proliferation and survival. The JAK-STAT pathway, the Akt-PKB pathway, and the MAPK pathway are important in cancer cells and are also targets of the cucurbitacin family.

The JAK-STAT pathway induces Janus-kinases (JACKs) and signal transducers and activators of transcription (STATs), and regulates cytokine and growth factor signals (Figure 2). In many cancer cells, constitutive activation of STAT3 and STAT5 has been known to play important roles in tumorigenesis [34]. After the initial finding by Blaskovich et al. that cucurbitacin I (JSI-124) is a dual inhibitor of STAT3 and JACK [16], many studies confirmed that cucurbitacin I is a powerful JAK-STAT inhibitor by blocking the tyrosine phosphorylation of STAT3 and JACK2 in various human cancers [33, 35, 36, 37, 38, 39]. However, cucurbitacin I did not affect other oncogenic signaling pathways, such as the Akt-PKB or MAPK/ERK pathways [35].

Furthermore, it has been discovered that cucurbitacin B inhibits the tyrosine phosphorylation of STAT3, STAT5, and JACK2 in pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) \textit{in vitro} and in Panc-1 xenografts \textit{in vivo} [32]. Inhibition of the JACK-STAT pathway affected various downstream targets involved in progrowth signaling (e.g., c-myc, cyclins, surviving) and apoptosis (e.g., p53, Bcl-xL, Bcl-2) [34, 36]. Therefore G2/M arrest and apoptosis in pancreatic cancer cells exposed to cucurbitacin B could be explained as a result of inhibition of the JAK-STAT pathway and was confirmed by the downregulation of p21\(\text{WAF1}\), cyclin A, and cyclin E, and upregulation of Bcl-xL. Like cucurbitacin I, cucurbitacin B did not inhibit other progrowth signaling pathways, such as the Akt/PKB and MAPK/ERK pathways in pancreatic cancer cells [32]. Interestingly, Sun et al. found that cucurbitacin A, which only differs with cucurbitacin B by its C-11 hydroxyl group, lost its activity as a STAT3 inhibitor while maintaining its activity as a JACK2 inhibitor [36], showing that the inhibition of STAT3 and JACK2 may follow different molecular mechanisms.

The anti-cancer mechanism of cucurbitacin in breast cancer cells is still not clear. Tannin-Spitz et al. exposed breast cancer cell lines (MCF-7 and MDA-MB-231) to cucurbitacin B/E glucosides and found that cucurbitacins increased the tyrosine phosphorylation of STAT3, unlike other cancers [33]. Considering the activated JAK-STAT pathway in breast cancer [34], this result
was contradictory. The authors hypothesized that concurrent inactivation of PBK in a cell type-specific manner might explain this unique regulation, which requires further research.

Considering the important role of STAT3 during inflammation [40], it is not surprising that part of the anti-cancer activity of cucurbitacins is linked to their anti-inflammatory activity. Chronic inflammation can make individuals predisposed to many types of cancer [40]. This seems to affect both cancer cells and normal macrophages through different mechanisms. In cancer cells, cucurbitacins work as STAT3 inhibitors, and make cells more susceptible to the attack of reactive oxygen species (ROS) and free radicals during inflammation [43]. In normal macrophages, however, cucurbitacins work as inhibitors of the IKK/NF-κB pathway rather than inhibitors of STAT3 [42, 43]. Inhibition of the IKK/NF-κB pathway by cucurbitacins results in the inhibition of key inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), whose overproduction contributes to tumorigenesis [41, 42, 43]. However, it is still not clear how cucurbitacins can selectively choose their target pathway depending on cell type.

Interestingly, when Escandell et al. used two human colon cancer cell lines without activated STAT3 (HCT116 and Hke-3), 23,24-dihydrocucurbitacin B and cucurbitacin R still suppressed tumour growth at a significant level [44]. Furthermore, the presence of active kRas in HCT116 cells showed more protection from apoptosis than Hke-3 cells, which do not have active kRas [25]. Since kRas is upstream of ERK, the result implies the effect of cucurbitacins on the MAPK pathway.

Indeed, the MAPK signaling cascade was another target that cucurbitacins acted upon. It has been shown that cucurbitacin B affects the MAPK pathway in glioblastoma (GBM) multiforme cells in vitro [31]. Cucurbitacin B-treated GBM cells showed an increased level of phosphorylated p38, phosphorylated JNK, and phosphorylated c-Jun, as well as a decreased level of phosphorylated ERK at the same time. Upregulation of JNK may induce apoptosis in GBM cells, and downregulation of p38 and ERK may block cytokine signaling. Chan et al. also showed that both phosphotyrosine-STAT3 and phospho-ERK were suppressed in the K562 leukemia cell line [45]. Increased JNK phosphorylation by cucurbitacin D in the Hep3B human hepatocellular carcinoma cell line was also reported [46].

The Akt-PKB pathway mediates signals from receptor tyrosine kinases (RTKs) and integrins. Currently, no cucurbitacins are known to inhibit the phosphorylation of Akt. Although Tannin-Spintz et al. showed the downregulation of PKB in breast cancer cell lines [33], further research is required to confirm the effect of cucurbitacin on the Akt-PKB pathway.

7. Synergistic effect of cucurbitacins with chemotherapeutic agents

Despite its excellent anti-cancer activity, clinical use of cucurbitacin has challenges to overcome, such as low therapeutic index and nonspecific toxicity. One of the solutions to these problems would be the use of cucurbitacins in combination, not only to enhance the efficacy of the treatment, but also to avoid the build-up of resistance in cancer cells. Moreover, some
drug combinations show strong synergism that helps to achieve the same therapeutic effect with a lower dose, and hence less toxicity. Encouragingly, some reports have shown that cucurbitacins show a synergistic effect with chemotherapeutic agents that are already established in the treatment of human cancers.

Saduka et al. showed that cucurbitacin E promotes cellular accumulation of doxorubicin, both by facilitating influx to and by preventing efflux from the tumour cells, implying synergistic effects of the two drugs [47]. Another study by Ramalhete et al. using cucurbitacin derivatives from *Momordica balsamica* confirmed statically the synergistic effect of some cucurbitacin derivatives and doxorubicin using the fractional inhibitory concentration index (FIX) [48]. Recently, the synergistic effect of cucurbitacin B with gemcitabine in pancreatic cancer was discovered [32]. Using an isobologram and combination index (CI) method, it was shown that in a certain concentration range, cucurbitacin B and gemcitabine showed a CI value less than 0.9, which showed synergism between two drugs *in vitro*.

Strikingly, cucurbitacin B induced no apparent toxicity *in vivo* [32]. As a single agent at high dose (1.0 mg/kg), cucurbitacin B induced a slight body weight loss after the first treatment, but no further toxicity was observed during the seven weeks of treatment. Any signs of toxicity after treatment were searched for using various immunohistochemistry stainings on major organs such as the liver, spleen, and kidney, as well as a blood and serum chemistry test. However, no signs of toxicity by cucurbitacin B were found. Considering the high dose of cucurbitacin B near LD<sub>50</sub> value (1.1 mg/kg) [26], the result was remarkable.

As Raikhlin-Eisenkraft et al. pointed out, many factors can affect the toxicity of cucurbitacins [25]. The bioreactivity of a compound can vary greatly depending on the presence of other compounds and the microenvironment *in vivo*. The discrepancy between the results and previous case reports can be partly explained by the extent of purity of cucurbitacins. Rapid advances in purification technology over the decades may have eliminated the other impurities coeluted with cucurbitacins from the plant source, which may be the true cause of toxicity in the past. Changes in the type of solvents for elution and dilution can also play a role. Defective immune system in athymic nude mice can be another possibility. For this reason, it was suggested that cucurbitacin toxicity in humans needs to be re-studied and should not be the reason to rule it out as a potential anti-cancer drug.

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<th>Effectiveness on cancer cell lines</th>
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### Cucurbitacin

<table>
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<td>Cucurbitacin R</td>
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<td>Colon: HCT 116 and Hke-3</td>
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**Table 2.** Cucurbitacin compounds from different plant species and their bioactivity on cancer cells.
Figure 2. Mechanism of activation of Janus kinases and signal transducer and activator transcription (JAK/STAT) pathway. Upon activator cytokine binding to its receptor on cell surface (e.g., IL-6 receptor), JAK/STAT pathway is activated (left) leading to sequential cell response. Inhibition signalling process by cucurbitacins indicate on JAK or STAT signalling.

8. Conclusions

This review has highlighted the interest or importance of some phytochemicals present in members of the Cucurbitaceae family, a group of plants used for a long time for many purposes such as ornamental, food, and medicinal. It is in this last area where their phytochemicals constituents have been pointed out as responsible for their medicinal properties. Flavonoids, carotenoids, sterols, oils, vitamins, and minerals are some of these phytochemicals, but is the terpenoid fraction known as cucurbitacins that has received special attention. This is because such compound has been associated as a potential cytotoxic compounds. More than ten years ago, a large number of cucurbitacins have been isolated and the use or applications for the treatment of different types of cancer was described. The review also described details about the mechanisms of how these phytochemicals act at the cellular level, but it seems that they are not specifically bound protein targets forming thioethers bonds through a Michael-type addition. This would allow these compounds to be conjugated with a broad array of potential proteins targets, many of which would be inhibited or disrupted as a consequence. As a result, their value as chemical biology probes is still limited and must be confirmed by independent means. The different findings of the reports reviewed also indicated that the binding mode of
cucurbitacins to protein targets means that optimization for selectivity would be unlikely to work, which would make it very difficult to reduce toxicities or improve the therapeutic window for future clinical applications. However, alternative ways that their potential therapeutic utility could be improved in the future is through targeted delivery to tumour cells, for example, through antibody-conjugation or incorporation in nanoparticles.

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References


